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DOCTOR OF MEDICINE

Airway challenges in different clinical phenotypes and their relationship to markers of disease and treatment

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Karine Leila Clearie

2010

University of Dundee

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Airway challenge in different clinical phenotypes and their relationship to markers of disease and treatment

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**Doctor of Medicine (MD)
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List of abbreviations

ACQ	Asthma control questionnaire
AHR	Airway hyper-reactivity
AMP	Adenosine monophosphate
AQLQ	Asthma quality of life questionnaire
ATS	American Thoracic Society
ATUE	Abbreviated therapeutic use exemption certificate
BDP	Beclomethasone dipropionate
BTS	British Thoracic Society
CFC	Chlorofluorocarbon
cNOS	Constitutive Nitric oxide synthase
COPD	Chronic obstructive pulmonary disease
CPET	Cardiopulmonary exercise test
CTA	Clinical trial application
DALY	Disability adjusted life year
ECP	Eosinophil cationic protein
EIA	Exercise induced asthma
EIB	Exercise induced bronchospasm
EMA	European Medicines Agency
EVH	Eucapnic voluntary hyperpnoea
FBT	Field based exercise test
FEF ₂₅₋₇₅	Forced expiratory flow
FE _{NO}	Exhaled tidal nitric oxide
FEV ₁	Forced expiratory volume in 1 second
FP	Fluticasone propionate
FPSM	Fluticasone/ salmeterol combination
FVC	Forced vital capacity
GINA	Global Initiative for Asthma
HDAC	Histone deacetylase
HFA	Hydrofluoroalkane
ICS	Inhaled corticosteroids
IgE	Immunoglobulin E
iNOS	Inducible nitric oxide synthase
IOS	Impulse oscillometry
LABA	Long-acting beta-2 agonist
LRTA	Leukotriene receptor antagonist
µg	Micrograms
N _{no}	Nasal exhaled nitric oxide
NO	Nitric oxide
OUCC	Overnight urinary cortisol creatinine clearance
PC ₂₀	Provocative concentration of methacholine required to cause a 20% fall in FEV ₁
PD ₁₅	Provocative dose of mannitol required to cause a 15% drop in FEV ₁
PEF	Peak expiratory flow
pMDI	Pressured meter dose inhaler
PNIF	Peak nasal inspiratory flow
R ₂₀	Proximal airway resistance
R ₅	Total airway resistance
R ₅₋₂₀	Peripheral airway resistance
RADS	Reactive airways dysfunction syndrome

RDR	Response dose rate
SEM	Standard error of the mean
WADA	World anti-doping agency
X5	Peripheral reactance

Declaration

I, KARINE CLEARIE, am the sole author of this thesis, and all the work is my own. The clinical research was carried out in the asthma and allergy research group, department of cardiovascular and lung biology, University of Dundee, Ninewells Hospital, under the clinical and educational supervision of Professor Brian Lipworth. I worked with two other research fellows in the department. Dr Peter Williamson and Mr Sriram Vaidyanathan. Clinical research technicians within the department ran the clinical trials. I was responsible for supervision of the studies in this thesis with cover from the other research fellows in periods of absence. The first study was designed by Dr Tom Fardon 4 years prior to my starting in the department. The principal investigator at the time was Professor Cathy Jackson, but I was responsible for dealing with the day-to-day management of this study, as well as submitting substantial amendments and boosting recruitment. I was also responsible for the data analysis and writing-up of this section of the trial. The second study was designed in collaboration with Professor Brian Lipworth and the team at AstraZeneca. The statistical team within AstraZeneca were responsible for analysing the study results. I was the named principal investigator for this study, and was responsible for trial supervision and writing up the final paper. I was principal investigator for all other studies, with full responsibility for study design, submission, data analysis and interpretation and write-up. This work has not been accepted for a research degree elsewhere.



Karine L. Clearie

Summary Statement

Asthma is a chronic disease, which affects over 300 million people worldwide. Despite the introduction of both national and international guidelines, asthma control remains poor. The aim of this thesis is therefore to explore possible new strategies for improving the management of asthma. The first strategy to be explored is 'titrating asthma treatment to suppress underlying airway inflammation'. The benefits of such a strategy have already been demonstrated, however, the lack of an adequate 'inflammometer' have limited its application to the research/ hospital setting. Mannitol challenge appears to be the most promising candidate, as it is portable and relatively cheap. The aim of the first study in this thesis is therefore to trial the use of mannitol challenge in a community setting. The selection of an appropriate 'inflammometer' is not limited to the clinical setting, it is equally important in research. This is particularly true when determining therapeutic equivalence between inhaled steroids. The aim of the second study in this thesis is therefore to determine which inflammatory outcomes demonstrate sufficient assay sensitivity, as part of a cross-over trial, to detect dose response effects on airway and systemic markers.

The second strategy to be examined in this thesis is tailoring asthma therapy according to asthmatic phenotype. Two groups of asthmatics that differ significantly from traditional 'inflammatory asthma' have been selected. Asthmatic smokers are known to develop relative resistance to the beneficial effects of inhaled steroids. A recent post hoc analysis of the GOAL trial has suggested that

smokers may gain greater benefit from the addition of a long-acting beta-2 agonist vs. doubling the dose of inhaled steroid. The third study in this thesis therefore aims to examine this in a prospective fashion. Another group of individuals in whom the traditional approach to asthma management and diagnosis may not be appropriate is elite athletes. It has been well documented that the mechanism of bronchospasm in athletes involves the drying/ cooling of airways. However, even within this category there are athletes to which this mechanism of action is unlikely to apply. Elite swimmers, for example, exercise in a warm, humidified environment. It therefore seems unlikely that tests designed to reproduce hyperosmolar shifts will have the same diagnostic sensitivity as they do in cold weather or track athletes. The aim of the fourth and fifth studies included in this thesis is therefore to compare various diagnostic tests in swimmers to determine which are the most sensitive.

Introduction

Burden of disease (incidence and prevalence of asthma)

Asthma is a chronic disorder of the respiratory tract which is thought to affect over 300 million people worldwide [1]. It has been estimated that this figure will increase exponentially over the coming years as communities adopt western lifestyles and become increasingly urbanised [2-4]. Urbanisation of the world's population is projected to increase from 45% to 59% in 2052, potentially leading to an additional 100 million new diagnoses of asthma worldwide [1].

The projected increase in prevalence of asthma is of concern due to the significant burden which asthma places on society [5]. In 1996 Peter Barnes published a review article examining this burden, which he sub-divided into direct, indirect and intangible costs [5]. He determined that the direct costs of asthma associated with medical care in the UK were between £322- £686 million [5]. 22% of this was attributed to physician care (75% to GP consultations, 25% to specialists), 37% to drug costs, and 25% to hospital costs (70-80% to in-patient care, and 14-18% to emergency care) [5]. 'Indirect' costs were more difficult to accurately quantify as they only occurred when asthma became sufficiently intrusive to interfere with lifestyle. These were associated with loss of productive work, premature retirement, time spent by others caring for sick relatives and premature death [5]. The GINA (Global Initiative for Asthma) Committee quantified this by ranking common disorders in terms of disability-adjusted life

years (DALYs), which combines information about mortality and morbidity in numbers of years lost. The number of DALYs lost due to asthma was estimated at about 15 million per year [1], ranking asthma as the 25th leading cause of DALYs lost worldwide. This is comparable with Diabetes Mellitus (23rd) cirrhosis of the liver (24th) and dementia (28th) [1]. 'Intangible' costs were associated with the impact of asthma on a patient's quality of life [5]. This was examined as part of the UK Action Asthma Survey, which collected qualitative data from 61234 asthmatic patients, through the provision of questionnaires in GP surgeries, pharmacies and outpatient clinics. They determined that 62% of patients felt that asthma had a moderate impact on their lives and 40% felt that it imposed a moderate restriction on their daily activities [6]. Jones et al examined the impact of asthma on an employed population using the St George's Hospital Respiratory questionnaire and found that up to 95% of respondents had an impaired quality of life compared to age matched controls [7].

National guidelines and the treatment of asthma

In 1994 the British Thoracic Society implemented the first national guidelines on the management of asthma [8]. Their aim was to decrease the mortality and morbidity associated with asthma by standardising its management. They set about doing this by reviewing the most up to date evidence based medicine and issuing recommendations. Current guidelines outline a stepwise management plan, beginning with short acting beta-2 agonists for the treatment of mild intermittent asthma at step 1 (BTS guideline), and culminating with the use of

oral corticosteroids and immunosuppressants at step 5. The aims of pharmacological management are the control of symptoms (including nocturnal symptoms and exercise induced symptoms), prevention of exacerbations and the achievement of best pulmonary function with minimal side-effects [8].

There is little doubt that inhaled corticosteroids (ICS) remain the treatment of choice for all severities of asthma. They are potent anti-inflammatory agents, which act in a relatively non-specific manner, inhibiting a wide variety of inflammatory cells, cytokines, and transcription factors [9]. These effects have been confirmed clinically in bronchial biopsy studies [10]. Evidence also suggests that early intervention with inhaled corticosteroid drugs may prevent any long term decline in lung function resulting from airway remodelling caused by untreated chronic inflammation [9]. The benefits of early treatment with ICS were confirmed by the START trial in which 7241 steroid naïve persistent asthmatic patients were randomised to receive either daily budesonide or placebo for 3 years. At the end of the study period, participants who had received budesonide had fewer severe exacerbations, fewer courses of oral corticosteroids, a longer time until their first exacerbation, and a greater number of symptom free days [11]. An earlier trial also found that mild-persistent asthmatics who received low-dose budesonide had fewer exacerbations and better symptom control, than patients receiving placebo [12]. These trials have firmly established the role of ICS in treating persistent asthma, however there is increasing concern over the side effect profile of ICS. For most adults with mild to moderate asthma, the

steep part of the dose-response curve for anti-asthma effects generally occurs at doses below 800 µg/day BDP (beclomethasone dipropionate) or equivalent [13]. In addition, the curve for systemic adverse effects becomes much steeper at doses above 800 µg/day, resulting in an inverted U shaped curve for the benefit to risk ratio [13]. This has been shown to be true, even in severe asthmatics. The main side effects associated with inhaled steroids include: adrenal suppression, osteoporosis, growth suppression, skin bruising, cataracts and ocular hypertension [13]. Current guidelines therefore recommend starting treatment with a relatively high dose for four to eight weeks in order to gain rapid optimal control, then gradually tapering the dose to determine the lowest effective maintenance dose [8].

Despite the publication of these guidelines and improvements in asthma treatment options, asthma remains a significant cause of mortality and morbidity for all age groups throughout the UK. This was echoed in the Asthma Insights and Reality Europe (AIRE) survey, which questioned asthmatic adults (n= 2083) from seven European countries about their symptom severity, healthcare use, exacerbation frequency, and perceived control [14]. Only 5.3% of all patients met all the criteria required for 'asthma control' as defined by the GINA guidelines [15]. 30.5% of adults complained of asthma-related sleep disturbance at least once a week, 27.9% had required an unscheduled urgent care visit over the last 12 months, and 57.2% of adults reported symptoms such as shortness of breath,

cough, chest tightness and wheeze at least once a month [14].

Mortality associated with asthma also remains a significant problem, and it has been estimated that approximately 1 in 250 deaths worldwide are due to asthma [1]. Mathers et al predicted, using mathematical modeling, that the global mortality rate from asthma will rise from roughly 240 000 in 2002 to between 320 000 - 402 000 by 2030 [16]. The most concerning aspect of this prediction is that mortality is mostly limited to those aged 15-59 [16]. Whilst new developments in pharmacological treatments do appear promising, none has made a radical impact on asthma mortality or morbidity. It is therefore clear that changes need to be made in the way we approach the management of asthma, in order to maximise the efficacy of treatments which are currently available to us. The aim of this thesis was to investigate this further:

Strategy 1) Titrating anti-inflammatory treatment to suppress surrogate markers of inflammation. In particular to identify which outcomes are most suitable in different clinical and research situations.

Strategy 2) Looking at different phenotypes of asthma rather than treating asthma as a homogenous disease.

Strategy 1: Inflammation

Airway inflammation is a salient feature of asthma. The link between asthma and inflammatory cells in the airways was first established during early case series of fatal asthma [17]. These described profound cellular infiltration of the respiratory mucosa associated with widespread mucus plugging [17]. More recently, Carroll et al compared the histological appearances of patients who died from asthma with those of non-asthmatic controls, and found a greater percentage of degranulated mast cells and increased numbers of neutrophils in the submucosal glands [18]. De Magalhaes Simoes et al described eosinophilic infiltration of the respiratory mucosa, extending from the nasal mucosa to the distal lung [19]. These results have been substantiated by a number of other studies, confirming that a diverse population of inflammatory cells including eosinophils, mast cells, neutrophils, and a variety of lymphocyte sub-sets are involved in the asthmatic process [20-22]. Interestingly, Carroll et al demonstrated that the distribution of inflammation within the lung varied with clinical severity [23]. Mild to moderate asthmatics were found to have predominantly central (i.e. large airways) infiltration, whereas patients with severe asthma had more distal disease [23].

Sputum eosinophil counts have been shown to increase in severe or poorly controlled asthma [24, 25] and after allergen challenge [26]. High sputum eosinophil counts have been shown to predict the development of asthma exacerbations [25, 27] and to predict failure of steroid reduction in clinically stable

asthmatics [26]. Green et al demonstrated that airway eosinophil counts decrease following treatment with inhaled corticosteroids and that this was linked with an improvement in symptoms and pulmonary function tests [28]. Reductions have also been noted following treatment with other anti-inflammatory agents such as theophyllines [29] and anti-leukotrienes [30].

Airway inflammation is known to correlate very poorly with subjective symptoms and measures of airflow limitation [21]. This is particularly true in mild to moderate asthmatics, in which unsuppressed airway inflammation may occur despite clinically stable disease, potentially leaving many patients with unchecked indolent inflammation [31]. This could result in frequent exacerbations and eventually irreversible damage due to airway remodeling.

Many studies have documented that asthmatic subjects have extensive structural changes in their airway [17, 32, 33]. These changes are present throughout the airway wall and the whole length of the bronchial tree and include basement membrane thickening, smooth muscle hypertrophy, abnormal deposition of matrix components, angiogenesis, proliferation of airway nerves and hypertrophy of glands [17]. Endobronchial biopsy studies have demonstrated that these changes, in particular reticular basement membrane thickness, are proportional to the number of inflammatory cells in the airways [34]. Autopsy studies have confirmed that extensive remodelling is present in both fatal and non-fatal asthma [35]. Laprise et al followed up 10 asymptomatic asthmatics and 10

control subjects with bronchial biopsy over a 2 year period. At baseline, groups had similar eosinophil counts, IgE levels and percentage predicted FEV₁. At 2 years, four had developed asthma and bronchial biopsies in these subjects revealed an increase in sub-epithelial fibrosis [31]. In a landmark trial Haahtela et al randomized 37 asthmatic patients to receive either 400µg budesonide or placebo in a double-blind manner for two years. A third group who had received terbutaline only for two years were crossed over in an open-label manner to treatment with 1200µg budesonide daily for the third year. They found significant differences between the placebo and budesonide groups in terms of FEV₁ and histamine PC₂₀. The condition of patients in the third group, who had initially been treated with terbutaline, improved, however, the degree of improvement appeared to be less than those who were treated with budesonide at the beginning of the three-year study. This suggests that early intervention with inhaled steroids is crucial when avoiding fixed airway obstruction [36]. These results were confirmed in the more recent Steroid Treatment as Regular Therapy (START) study, which involved 7,000 individuals (adults and children) with mild persistent asthma. The beneficial effects of ICS on accelerated decline of airway function over a 3 year period were established, as the rate of decline in post-bronchodilator FEV₁ was reduced by 22% in children and 42% in adults [11].

The benefits of a treatment strategy based on targeting inflammation were first demonstrated in 1999 by Sont and colleagues who carried out a parallel group study involving 75 mild to moderate asthmatics [37]. Subjects were randomised

to a treatment strategy aimed at reducing airway hyper-responsiveness (a surrogate of inflammation) or standard BTS management. Patients in the AHR group had a 1.8 fold lower rate of exacerbations and a greater improvement in their FEV₁ compared to the BTS group [37]. Bronchial biopsy specimens revealed that subjects in the AHR group also had a greater reduction in the thickness of their sub-epithelial reticular layer [37]. These findings were substantiated by Green and colleagues [38] who compared standard BTS management to titrating therapy with the aim of normalising induced sputum eosinophil counts. They found that eosinophil counts were 62% lower ($p=0.002$) in the treatment group, and that these patients suffered significantly fewer exacerbations and admissions compared with the BTS group [38]. The average steroid dose was the same in both groups [38]. These two studies have clearly demonstrated the benefits of a treatment strategy aimed at targeting surrogate markers of inflammation.

In order to accurately target inflammation a number of non-invasive surrogate markers have been developed. The pros and cons of each are outlined below:

Induced sputum

Induced sputum is probably the most obvious surrogate marker of inflammation. Sputum from asthmatic subjects is known to contain increased numbers of inflammatory cells such as eosinophils, which are strongly implicated in the pathogenesis of asthma [39]. Raised sputum eosinophil counts have been shown

to predict impending loss of asthma control, and to be a robust predictor of improvement in FEV1 following a course of oral corticosteroids [25, 40]. Leuppi et al reported that, at a cut-off point of 6.3%, sputum eosinophils had a sensitivity of 90% and a specificity of 63% for loss of control after stepwise reduction of inhaled steroid dose [41]. Using a cut-off point of 4%, Jones et al [42] reported a sensitivity of 59% and a specificity of 60% for loss of control after complete steroid withdrawal. In a landmark trial Green et al used these observations to titrate corticosteroid treatment against sputum eosinophil count [38]. Subjects in the eosinophil group had their treatment up-titrated if their counts were >3%. This led to a five-fold reduction in exacerbation rates compared to subjects titrated according to symptoms and measures of airflow limitation alone. Similar reductions were noted in other surrogate markers of inflammation such as FE_{NO}, and methacholine PC₂₀ [38]. However, no differences were seen in symptoms or any spirometric measure, lending further weight to the argument that these methods are inefficient at monitoring disease activity [38]. Interestingly, no significant difference was seen in inhaled steroid dose between the two groups, indicating that this novel treatment strategy does not owe its success to increased steroid dose [38]. The main drawback of induced sputum is that it is technically difficult, requiring expertise in sputum induction, sputum handling, cell counting and interpretation. Whilst the Leicester group have obtained consistently good results, with high sputum yields, other centres, such as ours, have time and again failed to do so. In addition, the technical expertise required to collect and process these samples make it an inaccessible and impractical test

for use in the community, limiting its use to specialist centres.

Peripheral blood eosinophil counts and ECP

An alternative method of quantifying eosinophil counts is to measure them in peripheral blood. It has been suggested that peripheral eosinophil count is representative the overall 'systemic burden' of the disease [43, 44]. High blood eosinophil counts have been shown to predict failure of corticosteroid withdrawal [45]. Eosinophil counts have also been shown to reduce following treatment with inhaled corticosteroids [44]. However, it has been clearly established that eosinophils exhibit greater sensitivity for assessing the anti-inflammatory response in sputum than they do in blood [44]. This suggests that activated eosinophils in the target tissue are more important than the total number in the blood stream, making measurement of blood eosinophil counts less applicable to use in routine clinical practice [44].

Eosinophil cationic protein (ECP) is a potent cytotoxic molecule that is released through the degranulation of eosinophils, and is thereby felt to indicate levels of eosinophil activation. It is therefore felt to be a more robust marker of asthmatic inflammation than total blood eosinophil count [46]. ECP levels in serum have been shown to correlate well with those in bronchiolar lavage fluid and sputum [47]. Studies looking at serum ECP in asthma indicate that levels are related to the severity of asthmatic disease, as measured by lung function and methacholine challenge [48]. ECP has been shown to rise following late phase allergen challenge and natural allergen exposure [49], and to decrease following

treatment with inhaled steroids [50]. However, ECP in sputum has been shown to be more sensitive than ECP in peripheral blood [44, 51]. Wever et al suggested that high ECP levels in serum can predict acute exacerbations, in spite of apparently satisfactory anti-inflammatory treatment [52]. However, these results conflict with those of Ferguson et al, who determined that ECP was a poor indicator of disease activity in chronic asthma, as it could not differentiate between bronchial and nasal inflammation [53]. Only one study has evaluated the efficacy of a treatment strategy based on ECP. In 2002, Lowhagen et al compared steroid titration according to serum ECP vs. pre-bronchodilator morning peak flow, and found no significant difference in exacerbation rates, symptom scores or FEV₁ between the groups [54]. These results suggest that although ECP is a useful clinical tool, it is best used in combination with other markers of disease activity.

Exhaled tidal nitric oxide

Nitric oxide (NO) is a molecule which is synthesised throughout the pulmonary epithelium and vascular tree by nitric oxide synthase (NOS) enzymes [55]. Two isoforms of NOS exist: the constitutive form (cNOS) which appears to protect the airways from excessive bronchoconstriction, and the inducible form (iNOS) which has a modulatory role in inflammatory disorders [56]. cNOS is produced in platelets, neuronal, epithelial and endothelial cells. It releases tiny quantities of NO (fM or pM) when airway receptors are stimulated by agonists such as acetylcholine and bradykinin [56]. iNOS is produced by neutrophils,

macrophages, epithelial, mesangial and vascular smooth muscle cells [56][58]. It releases large quantities (nM) of pro-inflammatory NO in response to up-regulation by immune cytokines, which may persist for hours or days [56]. NO has been detected in the exhaled breath of animals and humans by chemiluminescence [57]. Numerous authors have reported that the fraction of exhaled nitric oxide (FENO) measured in the expired breath of asthmatics is elevated compared to healthy controls. This has been linked to increased expression of iNOS [58], and is thought to reflect airway inflammation [59, 60]. FENO has been shown to increase during exacerbations and following exposure to allergen and viral infection [61, 62]. It correlates well with other markers of inflammation including sputum and peripheral eosinophil counts, ECP and airway hyper-responsiveness to methacholine [60, 63-67]. FENO has therefore been suggested as an aid in the diagnosis of asthma: A study by Berkman et al found it to have a similar diagnostic sensitivity to methacholine challenge, with a positive predictive value of more than 80%, however this finding was limited to steroid naïve patients [68]. Oral and inhaled corticosteroids have been shown to result in a rapid (6hrs after a single dose), dose dependent reduction in FENO [69-71]. Non-compliance or cessation of treatment with ICS will return FENO levels rapidly (3 to 5 days) to the pretreatment level [69]. This decrease is thought to occur due to a reduction in airway inflammation and an inhibitory effect on iNOS expression [55]. FENO has therefore been suggested as a marker of treatment response [72]. It has also been used to predict exacerbations, both spontaneous [61] and those induced by steroid reduction [25, 73], as high levels in treated

asthmatics have been found to herald a loss of clinical control [42]. Jones et al reported that a 60% increase in FE_{NO} between two visits provided a positive predictive value of 83% for loss of control after stepwise reduction of inhaled steroid dose [42]. More recently, Michils et al reported that changes in FE_{NO} in relation to asthma control (as measured by the Asthma Control Questionnaire) were prognostically helpful, as a single FE_{NO} measurement of >45 ppb excluded well-controlled asthma with a negative predictive value of 89% [74]. On the basis of repeated measurements, a 40% decrease in FE_{NO} was found to have a high positive predictive value (83%) and similar negative predictive value (79%) for a clinically relevant improvement in asthma control. A FE_{NO} reading consistently <30 ppb was associated with a low likelihood of exacerbation within 3 months. Van Veen et al reported that persistently high FE_{NO} readings are a predictor of accelerated decline in lung function in “difficult asthma” [75]. This suggests that serial monitoring could be used to predict and thereby prevent exacerbations. However, studies in which steroid dose has been titrated to suppress FE_{NO} have proved disappointing [76-79]. Smith et al randomized 97 asthmatic subjects to titration of their treatment based on either FE_{NO} or symptoms for one year. They failed to demonstrate a significant difference in exacerbation rates between the two groups, however there was a trend towards decreased exacerbations in the FE_{NO} group. In addition, the FE_{NO} cohort required significantly less inhaled steroids at the end of the study [78]. These results were confirmed by those of Shaw et al who carried out a similar study involving 118 asthmatic patients [77]. FE_{NO} is known to have a relatively shallow dose response curve, with a plateau

at approximately 400 μ g of BDP or equivalent, which may explain its limited use in upwards steroid titration [80, 81]. iNOS is also suppressed by cigarette smoke, limiting the application of FE_{NO} to a wider patient population [82]. FE_{NO} should also be used with caution as a diagnostic tool, as increased levels have been noted in other conditions such as bronchiectasis [83], rhinitis [84], and atopy [85]. Nevertheless, when interpreted appropriately FE_{NO} remains a useful tool in the management of asthma. Measurement of FE_{NO} is quick, painless, and non-invasive with a high degree of reproducibility and tolerability amongst subjects. It is easier to perform in a clinic environment than bronchial challenge, and the development of hand held analysers means that its use can be extended into a community setting.

Airway hyper-responsiveness and bronchial challenge

Airway hyper-responsiveness is a characteristic feature of asthma. It represents the tendency of the airways to constrict and narrow in response to irritant stimuli [86]. AHR has been shown to correlate well with airway inflammation on bronchial biopsy [34, 37], and with other surrogate markers of inflammation including FE_{NO}, sputum and blood eosinophil count, and ECP [60, 66]. AHR is known to vary over time, increasing during exacerbations and decreasing following treatment with anti-inflammatory medications. Sont and colleagues demonstrated the efficacy of a treatment programme in which ICS dose was titrated in order to suppress AHR [37]. They were able to achieve a 1.8 fold reduction in mild exacerbations compared to conventional steroid titration using symptoms and spirometry alone (0.23 and 0.43 exacerbations per year per patient respectively). The clinical improvement in the AHR group was accompanied by a significant reduction in sub-epithelial reticular basement thickness at the end of the two-year follow-up period [37].

AHR can be measured using either 'direct' or 'indirect' stimuli. 'Direct' stimuli include aerosols such as methacholine or histamine which act by stimulating specific receptors on the bronchial smooth muscle to cause bronchoconstriction [87]. 'Indirect' stimuli include adenosine monophosphate (AMP), mannitol, hypertonic saline, exercise and EVH. They provoke airway narrowing through the release of mediators from inflammatory cells and sensory nerves [88, 89]. These mediators then act on bronchial smooth muscle, causing it to contract and the

airways to narrow. The response to indirect stimuli is therefore dependent on the presence of inflammatory cells, and is thereby felt to be more reflective of airways inflammation than direct challenge. This theory was substantiated in a study by Van den Berge et al who demonstrated a stronger association between sputum eosinophil concentration and AHR to indirect stimuli, than direct stimuli [90].

The major advantage of indirect challenges is their capacity to act on many different cells causing the release of a wide variety of inflammatory substances (e.g. histamine, leukotrienes, prostaglandins, neuropeptides) [88, 91-94]. As ICS are known to cause a reduction in the number of inflammatory cells in the airway, it has been suggested that indirect stimuli, which act via these cells, may better reflect inflammatory status following treatment [95]. For example, taking budesonide (400–1000 µg) daily for 4–8 weeks has been shown to markedly inhibit responses to exercise [96-99], hypertonic saline [100-102], mannitol [103], and AMP [95, 104, 105]. A negative response to an indirect challenge suggests that inflammatory cells are not present in sufficient numbers to cause airway narrowing. In other words, the subject either does not have asthma or their asthma is currently under control with treatment. By contrast, it is highly likely that the same subject treated for the same time and with the same dose of steroid will remain hyper-responsive to inhaled histamine or methacholine [38, 101, 106, 107]. This suggests that direct stimuli may be more representative of airway 'smooth muscle stability' than inflammation per se.

Direct bronchial challenge (methacholine and histamine)

Direct bronchial challenges act by stimulating histaminic or cholinergic receptors on airway smooth muscle to cause bronchoconstriction. They are highly sensitive for clinically current symptomatic asthma and particularly useful to exclude current asthma, as they have a high negative predictive value. AHR, as assessed by direct challenge, increases following allergen exposure, both in the laboratory [108] and following natural [109] exposure. AHR has in turn been shown to be an important determinant of the airway response to allergen. The early asthmatic response to allergen is dependent on the degree of allergen sensitisation and the level of airway smooth muscle hyper-responsiveness [110-112]. Indeed, studies have shown that methacholine PC₂₀ can be used to predict the allergen PC₂₀ to within 3 doubling concentrations in 94% of cases [113].

Short-term within-subject repeatability studies (1-8 weeks) have shown that the 95% confidence intervals for repeat determinations of methacholine PC₂₀ lie within 1 doubling dilution [114]. Unlike other tests, methacholine challenge has a minimal significant difference equal to the variability of the test (i.e. 1 doubling dilution). The clinical significance of a change of this magnitude was clearly established by Sont et al who demonstrated that a difference of 0.64 doubling dilutions equated to a 1.8 fold reduction in exacerbation rates [37]. Responses to histamine have been shown to correlate closely with responsiveness to methacholine ($r^2 = 0.85$) [115]. Ward et al demonstrated significant improvements in methacholine PC₂₀ following treatment with regular inhaled

corticosteroids. This was accompanied by improvements in inflammatory cell count on bronchoalveolar lavage at 3 months, and a decrease in reticular basement membrane thickness at 12 months, compared with placebo [34]. Methacholine challenge has also been shown to demonstrate dose response following treatment with high and low dose corticosteroids [116].

Direct challenges have been found to have a sensitivity of only 57% for identifying people with asthma in a random population [117]. Their use is limited by their poor positive predictive value. A positive response can be seen in healthy people with no symptoms, smokers, congestive cardiac failure, and those with other diseases of the lung such as COPD, cystic fibrosis and bronchitis [118-121]. About 30% of patients without asthma but with allergic rhinitis have a PC₂₀ in the borderline AHR range [122]. The clinical significance of a positive methacholine challenge in the absence of symptoms has yet to be fully determined. Several possible explanations have been suggested [123]:

1. The patient has mild intermittent asthma but is a “poor perceiver” of their asthma symptoms
2. The patient never exercises or is never exposed to environmental triggers.
3. The mild AHR is due to a cause other than asthma (e.g. post-viral upper respiratory tract infection or cigarette smoking)
4. The patient has very mild or sub-clinical asthma that will become symptomatic in the future [36, 124]. Studies have shown that between 1.5

and 45% of asymptomatic patients with AHR develop asthma during 2-3 years of follow-up.

The pre-test clinical probability of asthma is therefore of vital importance in the assessment of results. Studies have also shown that the degree of AHR cannot be used to assess the clinical severity of asthma, as the correlation between the two is weak [125-127].

Methacholine and histamine act on specific receptors on the bronchial smooth muscle causing it to contract. Thus an important limitation in using one or other of these agents is that AHR to only a single mediator is tested. The involvement of many cells in the airway inflammation of asthma means that there are many substances to which the smooth muscle could respond. Furthermore, it is known that some inflammatory mediators (e.g., leukotrienes and prostaglandins) are more potent than either histamine or methacholine in causing airways to narrow [128, 129]. Thus failure to find AHR to one mediator would not exclude a positive response to another. It should be noted that a diagnosis of EIB is not excluded on the basis of a negative test to challenge with histamine or methacholine [129-131]. The most likely reason for this is that leukotrienes and prostaglandins [88, 93] are involved in the response to exercise.

Indirect challenge (AMP, mannitol, hyperosmolar saline, exercise and EVH)

Bronchial provocation tests (BPTs) have been used to assess patients with suspected AHR in clinical practice since the 1960s. Initially only direct aerosols, such as methacholine or histamine were used. However, in the 1970s, exercise as a BPT was developed for use in children [132]. It was recognised that most individuals with asthma experience symptoms when they exercise and that this is often one of the last symptoms to disappear following treatment with anti-inflammatory medications [133]. Studies investigating the pathophysiology behind the effects of exercise determined that water and heat loss from the airways were the major factors leading to bronchoconstriction [134]. Thus, the water content of the air inspired and ventilation rate were identified as the factors to be controlled to provoke exercise induced asthma (EIA). This led to development of eucapnic hyperventilation with dry air as a surrogate of exercise. Eucapnic hyperventilation was standardized by members of the U.S. Army and used to assess recruits for EIA, making testing fast, simple, and less expensive to perform than exercise [135]. The complex machinery involved and requirements for highly trained staff limited the applicability of this test outside the armed forces. Hypertonic saline was therefore suggested as an alternative, as it mimicked the osmotic shift that occurs following water loss from the airways. Later, an alternative osmotic bronchial challenge using a dry powder of mannitol was introduced [136]. The use of adenosine monophosphate (AMP) as a bronchial challenge has been developed over the last 15 years, however the

machinery and trained staff required limit its use to the research setting [137]. The airway response to AMP has many features in common with hyperpnoea and hypertonic stimuli. However, it differs in two specific ways: it is mediated via receptors (adenosine_{2b}) and appears to be mast-cell-specific, rather than being a stimulus to all cells in the airways, as the osmotic and thermal stimuli have the potential to be.

Indirect challenge tests are becoming increasingly recognised as useful tools for the monitoring of asthma following treatment with inhaled corticosteroids. The main advantage of indirect challenge over direct challenge is the lack of false positives: a positive response indicates that inflammatory cells and their mediators (prostaglandins, leukotrienes and histamine) are present in the airways in sufficient numbers and concentration to indicate that asthma is active at the time of testing. Healthy subjects respond with mean falls in FEV₁ of 2 to 6% [138-143] or small changes in conductance [144].

Mannitol challenge appears to be the indirect challenge test with the most potential as a tool to monitor or guide asthma therapy. It has been shown to correlate very closely with other indirect challenges including hypertonic saline, AMP, EVH and exercise [136, 145, 146], and aside from demonstrating very good repeatability, it requires little specialist equipment, other than a spirometer and dry-powder inhaler [136]. These factors make mannitol challenge relatively cheap to perform, portable and therefore ideal for use in the community.

However, despite this potential, no studies have been carried out utilising mannitol in a primary care setting.

Surrogate markers of inflammation and research

Whilst the identification of a suitable inflammatory surrogate is of critical importance in the clinical management of asthma, it is just as essential in the research setting. This is particularly true when establishing therapeutic equivalence between inhaled steroids. Traditionally, studies have utilised endpoints such as symptom scores, spirometry and peak flow. However, these are now known to be relatively insensitive measures for assessing response to ICS [147]. Surely it is fundamental to establish that supposedly equivalent inhaled steroids, whose clinical function is to suppress inflammation, do so to a comparable degree? We therefore need to determine which outcome measures provide sufficient assay sensitivity for detecting dose response effects on airway and systemic markers.

Strategy 2: Phenotype

The second alternative approach to asthma management, which will be explored in this thesis, is tailoring treatment/ diagnostic tools according to asthmatic phenotype. Current guidelines presume that asthma is a homogenous condition and only differentiate asthmatics on the basis of clinical severity. Asthma is in fact a very heterogeneous condition, which encompasses a wide range of clinical phenotypes. Preliminary studies have suggested that different subgroups respond differently to treatments and require different tests to diagnose and monitor treatment. We therefore propose that asthma treatment should be tailored to suit individual patients, or that at least subgroups should be identified, diagnosed and treated appropriately.

Smokers

Smoking is known to greatly increase the morbidity and mortality associated with asthma [148-150]. Asthmatic smokers are reported to have poorer symptom control [148, 150] an accelerated decline in lung function over time [149], more emergency department visits and hospitalisations [151] than non-smoking asthmatics. Cigarette smoke itself is a complex mixture of thousands of chemical compounds. It is thought to damage the airways in a number of ways, including direct toxicity to the epithelium, oxidative damage, and recruitment of inflammatory cells, especially neutrophils, to the airways [152, 153]. Smoking has been associated with increased airway inflammation and bronchial hyper-

reactivity (BHR) even in non-asthmatic individuals [154-156]. In addition, smokers have been found to develop relative resistance to the beneficial effects of both inhaled and oral corticosteroids [157-160]. For these reasons, most studies looking at asthma exclude smokers as a matter of course. Current asthma guidelines are therefore based almost exclusively on studies that exclude smoking asthmatics. Despite the logical expectation that people with asthma would avoid exposure to cigarette smoke, many studies suggest that the prevalence of active smoking among individuals with asthma is approximately the same as in the population at large. Current smoking rates among asthmatic patients have been reported as ranging from 17-35% [148, 150, 151, 161, 162]. We therefore feel that it is insufficient to assume that smoking cessation and/ or escalation of therapy are the only treatment options available to smokers. Studies to date have suggested that smokers may benefit from slightly different therapy to non-smokers. A recent subgroup analysis of the 'Gaining Optimal Asthma control (GOAL) study' [163] has revealed some interesting results. They discovered that smokers gained more benefit from FPSM vs. FP alone, as compared to non-smokers, in terms of reduction in exacerbation rates over 12 months. Jackson et al published a letter attempting to put the results of this study into a clinical context. They determined that in the majority of patients who were non-smokers it would take 25 years to obtain an additional benefit to prevent an exacerbation by taking fluticasone/salmeterol vs. fluticasone alone. In smokers it would take 6.66 years to see the same benefit conferred by using combination therapy [164]. The reasons for the apparent increased benefits of treatment with

FP/SM in smokers are unclear. A potential explanation for this could be that, in the face of the relative steroid resistance seen in smokers, the smooth muscle stabilisation conferred by the LABA becomes of greater significance. Another possible explanation is that smoking induces greater hyper-reactivity in the airway smooth muscle, which responds well to the smooth muscle stabilisation offered by LABAs. We would therefore like to investigate this further by comparing the benefit of FP/SM vs. FP prospectively in a cohort of smokers and non-smokers. We will also attempt to provide a mechanistic explanation by measuring both mannitol and methacholine challenge. Methacholine challenge is primarily a measure of airway tone, whereas mannitol is an indirect bronchial challenge and therefore thought to more accurately assess underlying airways inflammation.

Elite swimmers

Exercise induced asthma (EIA) is defined as a transient increase in airway resistance, which occurs following vigorous exercise [133]. Most people with known asthma will exhibit exercise-induced symptoms, however EIA has also been shown to occur in otherwise healthy people [165, 166]. It is particularly prevalent in athletes, with the highest prevalence reported in endurance and winter sports athletes (cross-country skiers 50%, ice hockey players 43%, swimmers 36%, summer and winter Olympic athletes 17%). This is thought to be due to a combination of high training loads and the training environment of the athletes. Two separate mechanisms have been proposed for this increased

prevalence. The first is the water loss/ humidity theory: increases in respiratory rate and increases in mouth breathing leads to drying of the respiratory epithelium. This drying results in changes in osmolarity of the pericilliary fluid, which in turn triggers mediator release, leading to bronchospasm [134]. The second theory is the heat exchange/ loss theory: heat loss from the bronchial tree in cold weather leads to reactive airways hyperaemia and mucous membrane swelling, which leads to the release of inflammatory mediators [167]. These increases in ventilation also substantially increase the exposure of the airways to cold air, allergens and pollutants; all of which may result in inflammation of the airways and therefore increased bronchial hyper-reactivity.

Whilst the above mechanisms can easily be applied to cold weather and endurance athletes, they cannot as easily be applied to elite swimmers, who exercise in a warm, humidified environment. Surprisingly, swimmers have one of the highest reported prevalence rates of EIB [168]. Swimmers have also been reported to have higher rates of rhinoconjunctivitis than other athletes [168]. The most commonly used method to achieve swimming pool hygiene in developed countries is chlorination. Exposing chlorine to organic material (saliva, sweat and urine) causes the release of toxic by-products such as nitrogen trichloride or trichloramine. These form a gaseous layer on the surface of the water, which is readily breathed in by swimmers. Recent reports have suggested that swimming in outdoor pools treated with chlorine products can predispose children to the development of asthma and recurrent bronchitis [169]. It has even been

suggested that the increasing prevalence of childhood asthma is related to the availability of swimming pools in Europe (due to airway epithelial damage from chlorine metabolites) [170]. Accidental acute chlorine exposure has been shown to induce neutrophilic airways inflammation with increased leukotriene B₄ levels in exhaled breath condensate [171]. Analysis of induced sputum in non-asthmatic elite swimmers has shown an increased proportion of eosinophils and neutrophils compared with healthy controls [172]. Asthmatic swimmers have also been found to have increased numbers of eosinophils and lymphocytes compared with healthy subjects and of neutrophils compared with asthmatic patients [168]. During a 5-year prospective follow up study Helenius and colleagues demonstrated persistent mild eosinophilic and lymphocytic airway inflammation in swimmers who remained active. Whereas in those who stopped training eosinophilic airway inflammation, bronchial responsiveness and clinical asthma attenuated and in some cases even disappeared [173]. Interestingly studies looking at F_{ENO} in children regularly attending swimming pools, showed no increase. F_{ENO} was also found to be normal in swimmers with high LTB₄ levels in exhaled breath concentrate [171].

Currently many asthma medications appear on the World Anti-Doping Association's (WADA) list of prohibited medications [174]. This regulation came into play in response to reports that beta2-agonists given systemically in large doses might influence skeletal and heart ventricular muscle fibres in research animals. However, these effects have never been demonstrated in athletes. The

use of many asthma medications is therefore only permitted if an “abbreviated therapeutic use exemption” (ATUE) certificate is granted. Failure to obtain such a certificate can result in a ban of up to 2 years. In order to obtain an ATUE certificate athletes are required to demonstrate objective evidence of airway narrowing [175]. A reliable test is therefore required to test for EIA.

A number of different direct and indirect provocation tests are available to test for EIA. Direct challenge tests such as methacholine and histamine have been shown to have a low sensitivity for EIA [176]. Indirect tests have been found to be significantly more sensitive. The current gold standard test is Eucapnic Voluntary Hyperpnoea (EVH), which requires subjects to hyperventilate whilst breathing in cold dry air, thereby replicating the conditions required to provoke EIB. However, as this test requires complex machinery, trained staff and is relatively expensive it is not widely used. Indeed it is only available in 3 centres in the UK. Alternative tests include exercise challenge tests (field and lab based) and osmotic challenges such as hypertonic saline and Mannitol.

Exercise tests can be performed either in the laboratory or in the field. Several studies have reported low sensitivity for EIA [177-179]. Dickinson et al [177] carried out a study comparing EVH and sport-specific exercise challenge testing (both lab and field). They found that 71% athletes had a positive EVH, whereas only 21% had a positive field test. None were positive to laboratory based exercise testing. The main problem with exercise testing is felt to be that trained athletes do not often reach high enough ventilatory rates to induce EIB (exercise-

induced bronchoconstriction). There is also no standardisation of cardiovascular workload and environmental conditions (temperature, humidity, presence of aeroallergens), so tests performed out of season may prove negative. The value of sport-specific exercise testing is yet to be established in swimmers, since the exact trigger for EIB in this group has not been fully determined. It may be that the specific conditions of the training environment (chlorine metabolites) play such a large role in inducing airways inflammation and bronchial hyper-reactivity that field based challenge will prove much more sensitive than in other sports.

Osmotic challenge tests such as hypertonic saline and Mannitol have been shown to be relatively cheap, easy to perform, and especially in the case of Mannitol practical for use at “point of need” [146]. In this regard mannitol, like exercise, acts via an osmotic mechanism. A positive response to Mannitol has been shown to identify individuals with EIB [146] and to have good repeatability [136]. A recent study by Holzer et al [180] which compared Mannitol challenge to EVH found mannitol to be highly sensitive (96%) and specific (92%) and have a positive predictive value of 92% and a negative predictive value of 96%.

A screening test should be sensitive, specific, and acceptable to the patient and should pick up a condition at a stage where it is easily treatable. We believe Mannitol challenge could be such a test. It is not currently known whether treating athletes with either symptomatic or asymptomatic EIA will translate into an improvement in performance. If this were the case we could postulate that identifying EIA early in an athletes’ career could lead to more young athletes

continuing to participate at a higher level. It may also allow current elite athletes to reach their full potential. Moreover identifying and treating EIA might lead to improvements in training efficiency.

We therefore propose to carry out a screening study to identify the prevalence of EIA in Scottish swimmers. We intend to use Mannitol challenge as our primary outcome and compare this to a sport-based exercise challenge. We plan to carry out this study during Scottish team training weekends. The exercise challenge will be performed when the swimmers have completed their “main set” which should be sufficient for them to reach the desired MVV (maximum voluntary ventilation) required to provoke EIB. We will also carry out nasal NO measurement and PNIF to identify rhinitis and measure FE_{NO} as another measure of airways inflammation.

Chapter 2: Methods

Detailed protocols for each study are described in individual chapters, however, many aspects of the methodologies are common to all studies and are therefore described in this section.

Airway measurements

Lung function

Lung function (FVC (forced vital capacity), FEV₁ (forced expiratory volume in 1 second), FEF₂₅₋₇₅ (forced expiratory flow between 25% and 75% of forced vital capacity)) were performed according to standard criteria laid down by the American Thoracic Society guidelines [181]. All measurements were carried out in triplicate, utilising a Micro Medical SuperSpiro (Micro Medical Ltd, Rochester, UK) spirometer, the highest of 3 values for FEV₁, repeatable within 5%, was recorded and the percentage predicted (according to ethnicity, height and weight) was calculated.

Mannitol challenge

Mannitol bronchial challenge was performed by administering mannitol gelatine capsules (Osmohale™ Pharmaxis Ltd, Sydney, Australia), inhaled from a dry powder device (Osmohaler, Pharmaxis Ltd. French's Forest, NSW, Australia) as previously described [136]. FEV₁ was measured 60 seconds after delivery of each dose (5, 10, 20, 40, 80, 160, 160, 160 mg). The test continued until the FEV₁ had fallen 15% (or there had been a >10% drop between two subsequent

doses) or the maximal cumulative dose of 635 mg had been administered. The provoking dose of mannitol to cause a 15% fall in FEV₁ (PD₁₅) was calculated by log linear interpolation. If a fall in FEV₁ of 15% had not been reached, a censored value of 1270mg was assigned.

Methacholine challenge

Methacholine was made up into doubling dilutions (concentrations ranging between 0.03- 32mg/ml) using benzyl alcohol. Methacholine challenge was performed according to recommended guidelines using a validated computer-assisted dosimetric method [182, 183]. An initial FEV₁ was taken before each challenge test to ensure safety (patients with an FEV₁ <60% predicted for age, gender and height were excluded from the challenge test). A further FEV₁ was taken following administration of the diluent (benzl alcohol) from which the % fall was calculated. Methacholine was then administered in doubling cumulative doses from 3-32mg/ml at 5-minute intervals, until a 20% fall from the post-diluent measure was recorded. The PC₂₀ values were calculated by computer-assisted log linear interpolation of the dose response curve [184]. If a fall in FEV₁ of 20% had not been reached, a censored value of 64mg/ml was assigned.

NO measurement

All participants underwent measurement of exhaled NO using either a NIOX (NIOX® Nitric Oxide Monitoring System, Aerocrine AB, Solna, Sweden) or a portable MINO (NIOX MINO® Airway Inflammation Monitor; Aerocrine AB, Solna, Sweden). The results of both devices have been shown to be directly comparable [185]. All measurements were made prior to measurement of spirometry to ensure accuracy of results. Participants were asked to avoid caffeine for 4 hours prior to measurement as this has been shown to cause a significant reduction in NO [186]. A sustained plateau of at least 8 seconds with a mouth flow rate of 50 ml/s and a pressure of 10 cm H₂O were used. The arithmetic mean was derived according to the current European Respiratory Society/American Thoracic Society recommendations [187].

Impulse oscillometry (IOS)

A Jaeger Masterscreen (Erich Jaeger, Hoechberg, Germany) was used to measure impulse oscillometry according to the guidelines [188]. Subjects supported their cheeks to reduce shunting, whilst impulses were applied for 30secs during tidal breathing. All manoeuvres were performed in triplicate and means taken [188].

Peak expiratory flow

All subjects were issued with a peak expiratory flow meter (Clement Clarke, Essex, UK) and diary, and instructed in their use. They were asked to record their peak flow every morning (prior to administration of asthma medication). Participants were instructed to carry out three technically correct PEF measurements, with a one-minute interval between assessments, and record the best of the three values.

Quality of life/ symptom measures***Juniper mini AQLQ and ACQ***

The mini juniper asthma quality of life questionnaire (Mini-AQLQ) [189] and asthma control questionnaire (ACQ) [190] were used in several of the studies. They have been repeatedly validated, and have been shown to be sensitive measures of quality of life and control in asthma. The mini-AQLQ has a total of fifteen questions, each of which has a response scored out seven. The questions are then grouped into four domains: activity limitations, symptoms, emotions, and exposure. Each group is then averaged to obtain a score for each group. A change in score of greater than 0.5 is considered clinically relevant [191].

Symptom scores

Symptom scores varied from study to study, the method for each will be described in the individual methods sections.

Skin prick testing

Skin prick testing to eight common aeroallergens (grass, tree, weed, house dust mite, aspergillus, feathers, cat and dog) was carried out in several of the studies. Subjects were asked to withhold antihistamines and leukotriene receptor antagonists for at least 4 days prior to the test. Allergen drops (Bencard Testing Solutions: Welwyn Garden City, UK) were applied to the forearm; they were then pressed into the skin using disposable lancets. A positive response was defined as any wheal with a diameter that was 3mm greater than the negative control, at least 15 minutes after skin prick.

Blood and Urine collection***Overnight urinary cortisol creatinine clearance***

Subjects were instructed to empty their bladder at 10pm then collect all subsequent urine produced overnight for analysis (10pm-8am). They were also asked to provide an early morning (8am) spot sample. This sample concluded their overnight sample and as such was included in their overnight sample, however the 8am sample was also analysed separately. 5mls from both samples were stored in a freezer at -20°C throughout each individual study and analysed

in batches on completion. The urinary cortisol was measured using a commercial radioimmunoassay kit (DiaSorin Ltd, Wokingham, Berkshire, UK), which has no cross reactivity with fluticasone. The intra assay coefficient of variation was 4% and the inter assay coefficient of variation was 8%. Urinary creatinine was measured on a Cobas-Bio auto analyser (Roche Products, Welwyn Garden City, UK). The intra assay and inter assay co-efficient of variation was 4.6% and 3% respectively.

Blood eosinophil count and Eosinophilic cationic protein (ECP)

Blood samples for eosinophils and ECP were taken prior to performing bronchial challenge. For the measurement of ECP, whole blood was allowed to rest for 60 minutes at a temperature of 22-24°C prior to being centrifuged for 10 minutes at a speed of 3000rpm (at 4°C). The supernatant was then stored in a freezer at -20°C and analysed in batches on completion of the study by the departmental laboratory. It was measured using an enzyme linked immunoassay technique (UniCAP; Sweden Diagnostics UK Ltd, Milton Keynes, UK) with an intra-assay co-efficient of variation of 3.3%. Blood eosinophil count was analysed by the Ninewells Hospital haematology laboratories using a Sysmex XE 2100 Hematology auto analyzer.

Quality control

Sensitivity, specificity and coefficients of variance were checked for each batch of assays carried out in-house. Eosinophil counts, measured in the Ninewells haematology lab were subject to NHS standards of quality.

Chapter 3:

Establishing the role of surrogate markers of inflammation in clinical and research settings

Part a: supervised step-down of inhaled steroids in a community setting

Study aims: To determine the effect of stepping down inhaled steroids in a community setting on surrogate markers of inflammation.

Supervised Step-down of Inhaled Corticosteroids In the Community

Introduction

Inhaled corticosteroids (ICS) are the first line anti-inflammatory therapy in the treatment of asthma [192], however higher doses are associated with local and systemic adverse effects [13]. Current asthma guidelines recommend stepping down of steroid dose once asthma control has been achieved [8]. There is little evidence to suggest the best method to step down treatment, or to ensure the safety of this approach. Hawkins and colleagues reduced the ICS dose in a range of asthmatic patients by a mean of 25% without a significant rise in asthma exacerbations or change in measures of health status [193]. They did not, however, examine surrogates of inflammation or airway hyper-responsiveness (AHR). It is recognised that symptoms correlate poorly with underlying inflammation [21]. Studies have demonstrated that titrating ICS to suppress surrogates of inflammation leads to reduced exacerbations and decreased airways remodelling [37, 38]. During the initial run-in phase of an ongoing community based clinical trial, ICS doses were stepped down to determine the lowest dose required to achieve stability. During this phase inflammatory surrogates including FE_{NO} and AHR to mannitol were measured, in addition to lung function and quality of life. Mannitol was selected because it is portable and easy to use in a community setting. In addition, AHR to mannitol has been shown to be a good predictor of failure of ICS step-down [41].

Methods

Participants

119 eligible patients were recruited from 35 general practices throughout Tayside. This paper presents data obtained during the step-down phase of a large community based study. Participants were required to be aged 16 years and above, non-smokers, have a diagnosis of persistent mild to moderate asthma, be clinically stable, and currently treated with ≥ 400 μg beclomethasone dipropionate (BDP) equivalent. At point of entry into the study, patients were required to demonstrate AHR to mannitol challenge in terms of a 10% fall in FEV₁ to a dose of mannitol of 635 mg or less. Exclusion criteria were: oral steroid in the preceding three months; aspirin intolerance; FEV₁ $\leq 50\%$ predicted; pregnancy; recurrent lower respiratory tract infections; and the presence of concomitant respiratory disease such as bronchiectasis. Patients who failed to become unstable on 200 μg BDP equivalent, or who were unable to step below 800 μg were withdrawn, as these were entry criteria for the subsequent study. The study was approved by the Tayside Committee on Medical Ethics (CTA, MF8000/13398), and all participants gave written informed consent.

Protocol

Patients underwent systematic step-down of their medication with two weekly follow up. At screening patients were issued with a peak expiratory flow (PEF) and symptom diary card and asked to monitor their PEF for two weeks: this served as their baseline for the study. Additional asthma therapies (leukotriene receptor antagonists (LTRAs) or theophyllines) were discontinued. Patients on combination inhalers were switched to an equivalent dose of inhaled steroid only. The dose of inhaled steroid was then halved every two weeks till patients were on 200µg BDP equivalent or became clinically unstable. Clinical instability was defined as: diurnal variation in domiciliary PEF $\geq 20\%$; deterioration in FEV₁ $\geq 20\%$ from baseline; mean use of inhaled reliever medication ≥ 0.5 puffs daily from baseline; an increase in symptom scores of ≥ 0.5 daily from baseline. (The asthma symptom score asks for a number analogue of symptoms from 0 to 3, where: 0 is symptom free; 1 is minimal symptoms; 2 is moderate symptoms which may limit activity; 3 severe symptoms which limit activity). Once unstable, participants stepped back up to the last stable dose of ICS (this was designated the 'lowest dose required for stability'). FE_{NO} and AHR were recorded at the start and end of the step-down, while asthma quality of life questionnaires and spirometry were measured throughout the step down period.

Lung function

Lung function (FVC (forced vital capacity), FEV₁ (forced expiratory volume in 1 second)) was measured using a Micro Medical SuperSpiro (Micro Medical Ltd, Rochester, UK) spirometer according to the American Thoracic Society guidelines [181]. The highest of 3 values for FEV₁, repeatable within 5%, was recorded and the percentage of percent predicted was calculated.

Mannitol challenge

Mannitol bronchial challenge was performed by administering mannitol gelatine capsules (Osmohale™ Pharmaxis Ltd, Sydney, Australia), inhaled from a dry powder device (Osmohaler, Pharmaxis Ltd. French's Forest, NSW, Australia) as previously described [136]. FEV₁ was measured 60 seconds after delivery of each dose (5, 10, 20, 40, 80, 160, 160, 160 mg). The test continued until the FEV₁ had fallen 10% or the maximal cumulative dose of 635 mg had been administered. The provoking dose of mannitol to cause a 10% fall in FEV₁ (PD₁₀) was calculated by log linear interpolation.

NO measurement

The measurement of FE_{NO} pre and post step down was introduced, as part of a substantial amendment, after recruitment had commenced. Consequently, 83/119 participants underwent measurement of FE_{NO} using a portable MINO (NIOX MINO® Airway Inflammation Monitor; Aerocrine AB, Solna, Sweden). FE_{NO} was measured during a sustained plateau of at least 8 seconds with a

mouth flow rate of 50 ml/s and a pressure of 10 cm H₂O following a tidal breath. The arithmetic mean was derived according to the current European Respiratory Society/American Thoracic Society recommendations [194].

Mini juniper quality of life questionnaires (Mini-AQLQ)

At each study visit each patient completed the Juniper mini-AQLQ [189].

Statistical analysis

SPSS version 15 (SPSS Inc, Chicago, Illinois) was used to perform the statistical analysis. Normality of data was assessed using the Kolmogorov-Smirnov; non-Gaussian data were log transformed prior to analysis. Gaussian data were assessed using paired Student's t-tests. ICS dose and AQLQ pre- and post-step down was analysed using Wilcoxon Signed Rank Tests. A probability value of less than 0.05 (two tailed) was considered significant.

Results

Demographics

One hundred and fifty patients entered the step down phase of the clinical trial. One hundred and nineteen (49% female, 52% male) of these were randomised and entered the subsequent clinical trial. Thirty one patients withdrew during the

step-down phase: 1 failed to become unstable when cut back to 200µg ICS; 2 became unstable on ≥ 800 µg ICS; 3 were unable to discontinue long acting beta agonist therapy; 2 were withdrawn due to failure to comply with the protocol; 6 withdrew due to personal reasons; and 17 were lost to follow-up (Figure 1). The mean (SEM) age of participants who dropped out was 57 years (2) compared with 52 years (1) in those who completed step-down. Drop-outs also had a significantly higher mannitol PD₁₀: 184.5 mg (95 % CI 113.5 – 299.8) compared with 100 mg (95 % CI 76.2 – 131.2), $p=0.036$. Other baseline characteristics did not differ significantly between the two groups (table 1).

Table 1: Baseline characteristics of drop-outs vs. those who completed.

Variable	Completed	Drop-outs	p-value
BDP equivalent ICS dose (mcg/day)*	400 (400-800)	800 (400-1000)	0.055
Mannitol (PD ₁₀ mg) †	99.99 (76.2 to 131.2)	184.47 (113.5 to 299.8)	0.036
FE _{NO} (ppb) †	26.79 (22.1 to 32.5)	17.83 (9.9 to 32.0)	0.144
Spirometry ‡			
FEV1 % predicted	86.16 (1.512)	90.13 (3.396)	0.244
FVC % predicted	91.56 (1.376)	95.13 (3.215)	0.254
PEF % predicted	90.20 (1.695)	91.61 (3.910)	0.713
Age	52.00	57.83	0.049
Sex (F:M)	48.7%: 51.3%	62.1%: 37.9%	

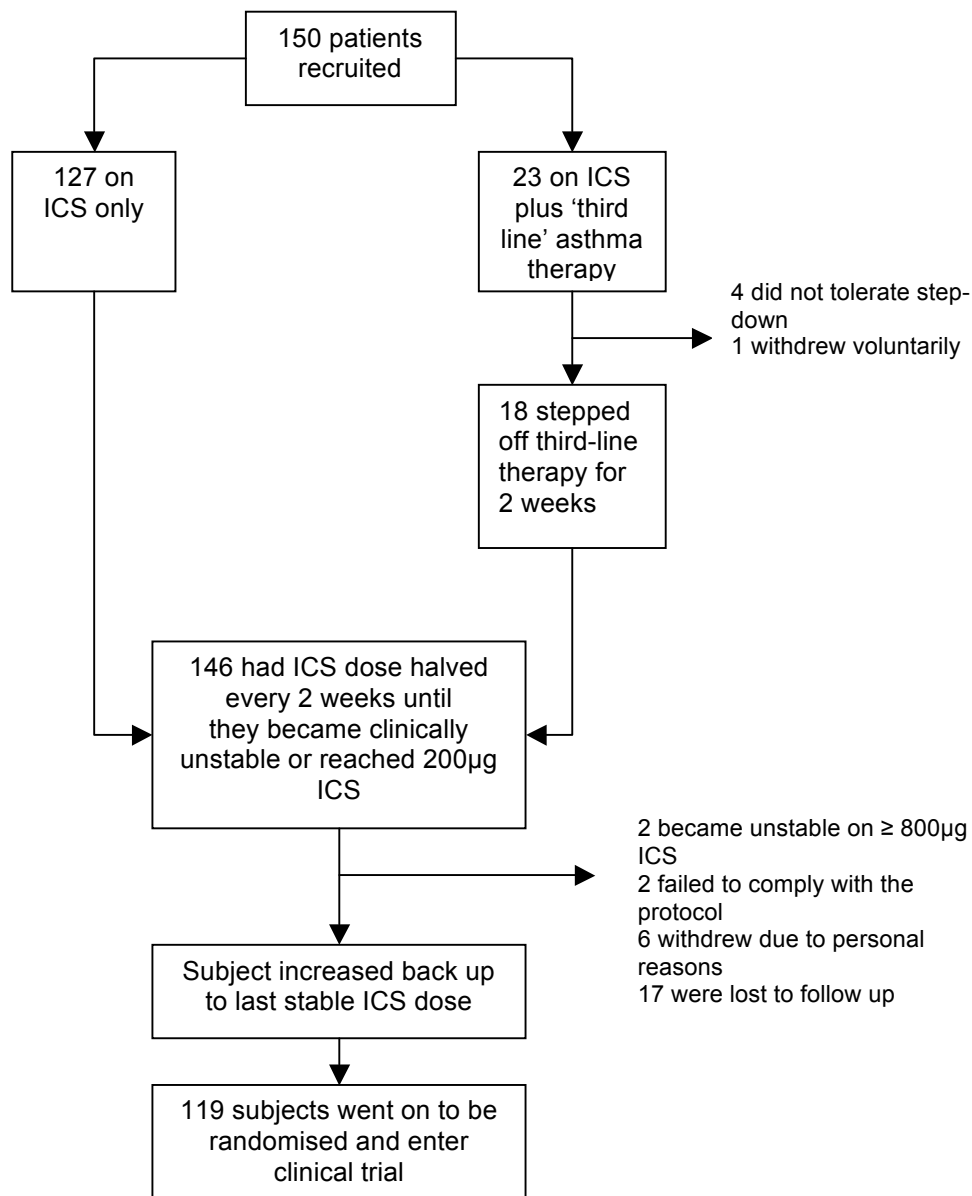
Abbreviations: ICS, inhaled corticosteroids; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; PEF, peak expiratory flow rate; PD₁₀, provocative dose of mannitol causing a 10% decrease in FEV1.

Data presented as arithmetic mean (SEM) unless otherwise indicated.

*Expressed as median (inter-quartile range)

† Shown as geometric mean (95% CI)

‡ Expressed as percentage of that predicted by age, sex, and height, mean (SEM).

Figure 1: step down procedure

Withdrawal of medication

23 participants were taking additional third line asthma therapy (long-acting β 2-agonists (n=21), theophyllines (n=1) or leukotriene receptor antagonists (n=1)) at the start of the step down. Only four participants did not tolerate withdrawal of their third line therapy; one withdrew voluntarily. In patients who completed step-down, the median (interquartile range) dose of BDP or equivalent was 400 μ g (400 – 800) at baseline, compared with 250 μ g (200 – 400) post step-down, giving a median reduction of 150 μ g ($p < 0.0001$) (Figure 2). Subjects underwent an average of 1.49 (0.07) step-downs prior to either becoming unstable or reaching 200 μ g BDP equivalent.

Figure 2: median change in ICS dose (μ g) pre and post step-down

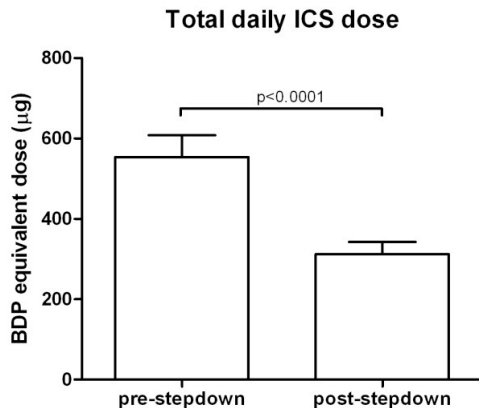


Table 2: Baseline and post step-down values

Variable	Baseline	Post step-down	P value
BDP equivalent dose (µg/day)*	400 (400-800)	250 (200-400)	<0.0001
FE _{NO} (ppb) †	26.9 (21.9 – 32.4)	28.2 (23.4 – 33.1)	0.43
Spirometry‡			
FEV1 % predicted	86.16 (1.51)	84.46 (1.46)	0.04
FVC % predicted	91.56 (1.38)	91.52 (1.44)	0.97
PEF % predicted	90.20 (1.70)	90.02 (1.56)	0.87
AQLQ			
Activity limitation	5.22 (0.11)	5.65 (0.10)	<0.0001
Emotional function	5.18 (0.13)	5.68 (0.11)	<0.0001
Symptoms	5.44 (0.11)	5.79 (0.10)	<0.0001
Environmental stimuli	5.40 (0.11)	5.82 (0.10)	<0.0001
Total score	5.44 (0.10)	5.83 (0.09)	<0.0001

Abbreviations: ICS- inhaled corticosteroids; FEV1- forced expiratory volume in 1 second; FCV- forced vital capacity; PEF- peak expiratory flow; PD10- provocative dose of mannitol causing a 10% decrease in FEV1

Data presented as arithmetic mean (SEM) unless otherwise indicated. Step-down measures were taken 2 weeks after reduction of steroid.

*Expressed as median (inter-quartile range) † Shown as geometric mean (95% CI). ‡ Expressed as percentage of predicted for age, sex and height.

Inflammatory Surrogates

The geometric mean (95 % CI) mannitol PD₁₀ was 100.0 (76.2 to 131.2) at baseline; and 137.2 (104.5 to 180.1) post step-down. This represents a 0.46 doubling dose difference for pre vs. post step-down (95 % CI 0.02 - 0.89), p=0.04. The geometric mean response-dose-rate (%fall in FEV1/ maximum dose mannitol given) was 1.73 (1.71 to 1.75) at baseline and 1.95 (1.93 to 1.96) post step-down, p=0.73. Data for change from baseline in FE_{NO} was available for 83/ 119 subjects. The geometric mean fold ratio for pre vs. post in exhaled FE_{NO} was 0.96 (0.87 - 1.06), p=0.43.

Figure 3: Shift in mannitol challenge post step-down

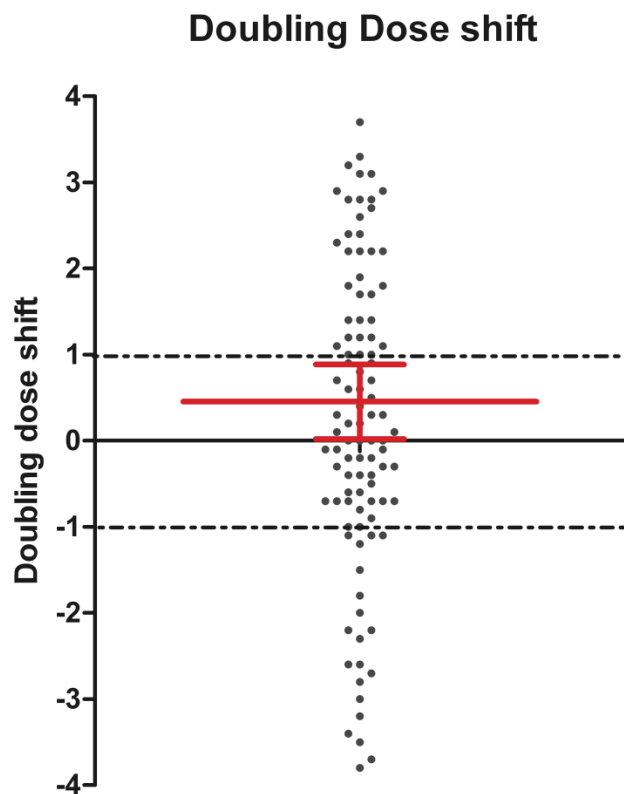


Fig 3. Doubling dose shift (i.e. post step-down PD_{10} minus pre-step down PD_{10}) in mannitol for each individual patient. The intra-individual biological variability of mannitol challenge is ± 1 dd, so individuals lying between +1 and -1 (depicted by interrupted line) can be assumed to show 'no change' in mannitol challenge post step-down (47%). Individuals with $> +1$ dd demonstrated an improvement in mannitol challenge (34%). Those with a < -1 dd shift demonstrated a worsening in mannitol challenge (19%).

Table 3: Baseline and post-step-down data divided by change in mannitol challenge post step down

	> +1 doubling dose change "Improvement" (n=37)			+/- 1 doubling dose change "No change" (n=62)			< - 1 doubling dose change "Worsening" (n=20)		
	Pre	Post	Change from BL	Pre	Post	Change from BL	Pre	Post	Change from BL
Mannitol PD ₁₀ (mg)‡	47.8 (1.3)	328.2 (1.2)	-2.8 (0.3)* †	137.1 (1.2)	131.5 (1.2)	0.1 (0.1) †	180.6 (1.3)	30.3 (1.3)	2.6 (0.2)* †
FE _{NO} (ppb)	33.8 (6.0)	31.3 (4.0)	-2.6 (3.7)	39.6 (5.2)	38.3 (5.4)	0.9 (1.5)	33.0 (8.4)	36.7 (7.7)	3.7 (6.0)
Spirometry									
FEV ₁ % predicted	86.4 (2.5)	84.4 (2.8)	-2.0 (1.3)	85.3 (2.3)	84.4 (2.1)	0.9 (1.5)	88.5 (3.6)	84.8 (3.5)	-3.7 (1.8)
FVC % predicted	91.2 (2.2)	91.2 (2.9)	-0.03 (1.7)	93.7 (2.1)	93.1 (2.1)	0.6 (1.4)	90.4 (4.0)	89.5 (3.6)	-0.9 (2.8)
PEF % predicted	88.1 (2.8)	90.0 (2.7)	1.9 (1.8)	89.8 (2.8)	89.3 (2.4)	0.5 (2.1)	94.8 (3.6)	91.8 (3.3)	-3.0 (1.8)
BDP equivalent ICS dose (mcg/day)	622.9 (52.2)	350.0 (35.6)	-272.9 (42.1)*	634.7 (56.8)	338.8 (27.4)	-295.9 (50.0)*	752.6 (115.6)	397.4 (46.0)	-355.3 (104.4)*
AQLQ									
Activity limitation	4.6 (0.4)	4.2 (0.4)	-0.4 (0.4)	4.4 (0.4)	4.5 (0.4)	0.0 (0.3)	4.2 (0.5)	4.5 (0.6)	0.4 (0.2)
Emotional function	4.9 (0.3)	4.4 (0.5)	-0.4 (0.4)	4.7 (0.4)	4.5 (0.4)	-0.1 (0.4)	4.2 (0.5)	4.5 (0.6)	0.3 (0.1)
Symptoms	4.7 (0.3)	4.3 (0.5)	-0.4 (0.3)	4.4 (0.4)	4.4 (0.4)	0.0 (0.3)	4.2 (0.5)	4.3 (0.5)	0.2 (0.1)
Environmental stimuli	4.8 (0.3)	4.4 (0.4)	-0.4 (0.4)	4.5 (0.4)	4.6 (0.4)	0.0 (0.3)	4.4 (0.5)	4.6 (0.6)	0.2 (0.1)
Total score	4.9 (0.3)	5.2 (0.3)	0.3 (0.1)*	4.7 (0.3)	5.1 (0.3)	0.4 (0.1)*	4.6 (0.5)	4.8 (0.5)	0.2 (0.1)

†Change from baseline presented as doubling dilutions

* Significant change from baseline (P<0.05)

Abbreviations: ICS, inhaled corticosteroids; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; PEF, peak expiratory flow rate; PD₁₀, provocative dose of mannitol causing a 10% decrease in FEV₁; BL, baseline.

Data presented as arithmetic mean (SEM) unless otherwise indicated. ‡ presented as geometric mean (SEM). Baseline values are shown after washout before each randomised treatment period. Post step down measurements were made 2 weeks after the final dose reduction.

Table 4: Individual data for subjects who had a worsening in dd-shift post step-down

Subject no	DD shift	Change from baseline				Total AQLQ score	Drug dose (mg)
		FEV1 (% predicted)	FVC (% predicted)	PEF(% predicted)	Change in FE _{NO} (% change)		
2	-2.7	-17	-14	-16		0.7	200
7	-3.5	-2	1	-9		0.7	750
8	-2.2	-7	-7	-7		0.1	200
11	-1.5	-13	-5	-3		-0.3	0
13	-2.8	-9	-7	-2		0.0	200
30	-2.3	-7	9	-2		-0.3	0
31	-2.0	-3	4	-9		0.1	400
32	-1.2	4	8	-2		0.4	250
36	-3.4	8	29	2		0.7	0
53	-1.1	-5	-9	0	70.6	0.0	200
54	-1.1	0	-6	-3	79.3	0.0	400
56	-4.9	15	24	0	28.6	-0.4	1750
57	-1.8	5	-3	13	0.0	-0.4	500
62	-3.7	-8	-12	-22		0.3	1250
78	-3.2	4	-15	5		0.0	200
79	-2.6	-12	-4	2	-34.8	0.9	0
80	-2.6	-13	-9	4	-34.2	0.0	0
91	-3.0	-6	-5	-13	2.0	0.9	200
110	-3.8	0	15	4		0.5	250
147	-2.2	-8	-12	-2	59.0	0.1	200
Mean	-2.58	-3.65	-0.90	-3.00	21.21	0.19	347.50
(SEM)	(0.22)	(1.78)	(2.75)	(1.78)	(16.03)	(0.09)	(99.31)

Table 5: Individual data for subjects who had an improvement in dd-shift post step-down

Subject no	DD shift	Change from baseline				Total AQLQ score	Drug dose (mg)
		FEV1 (% predicted)	FVC (% predicted)	PEF(% predicted)	Change in FE _{NO} (% change)		
40	1.1	7	6	8	45.0	0.5	600
4	2.2	-14	-13	33		-0.1	0
10	1.4	-1	-9	-2		0.2	1000
16	1.2	-7	-13	-2		0.5	200
18	1.2	-7	-8	-6		1.4	0
23	2.9	-5	-5	11		-1.4	200
24	1.8	-4	-2	-10		0.1	200
25	6.2	-16	3	-11		0.5	200
26	6.5	0	-6	0		1.3	200
33	2.3	-10	13	-10		-0.8	600
34	1.4	-1	8	12		0.7	400
35	1.8	8	17	3		-0.3	200
37	5.9	1	5	-5	50.0	0.1	200
39	7.8	19	10	18		1.1	400
42	3.2	-8	3	11			0
43	2.4	3	2	1	-31.0	0.1	600
46	3.1	-2	-2	16	-37.5	0.3	200
46	3.1	-2	-2	16	-37.5	0.0	200
47	3.7	-12	-17	-10	-14.3	-0.7	200
50	1.4	1	-5	21	16.1	0.8	0
58	1.2	-4	-1	1	-21.1	-0.8	200
61	2.8	-5	-5	-8		1.5	600
64	2.2	1	2	3	11.1	2.1	0
65	1.7	2	0	-2	-52.9	0.0	500
67	2.4	-2	-4	-3	10.0	0.3	400
72	1.7	0	-5	-2	17.5	0.2	200
74	1.9	4	29	-5	-7.9	0.9	200
82	5.7	5	4	22		0.1	400
90	2.9	-4	2	10	-16.7	0.2	250
92	2.8	-1	1	3	-20.0	0.3	200
99	2.6	10	6	-3	35.7	1.3	200
100	2.2	-5	-3	-11	46.7	0.6	0
106	2.2	-23	-21	-12	26.7	-0.1	0
108	3.3	-9	-3	-11	-59.2	0.3	400
117	2.7	9	28	-6	64.7	1.0	0
120	2.8	9	-2	2	3.6	-0.3	0
121	1.1	-12	-14	-2	-29.7	0.2	800
Mean (SEM)	2.78 (0.27)	-2.0 (1.3)	-0.03 (1.73)	1.89 (1.80)	13.45 (11.63)	0.33 (0.12)	268.92 (39.86)

Figure 4: Shift in FE_{NO} post step-down

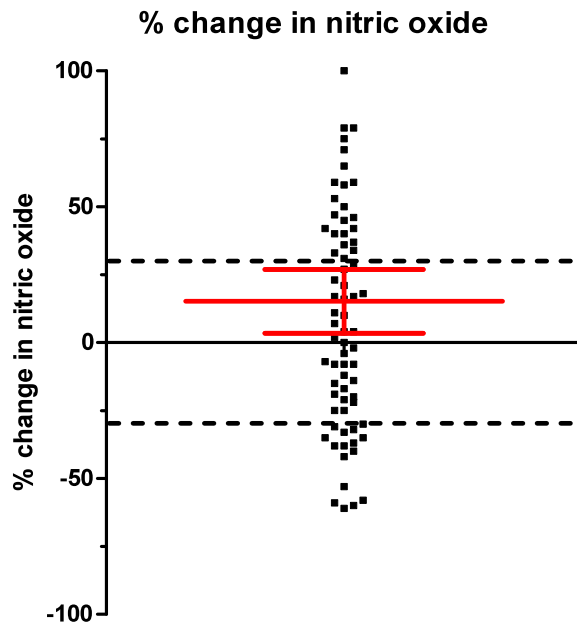
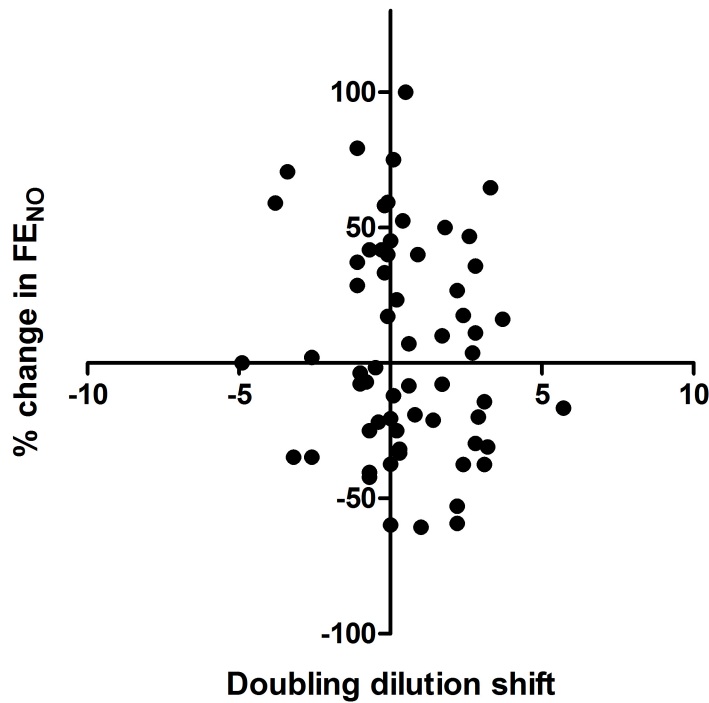


Fig 4. Change in exhaled nitric oxide (i.e. post step down - pre step down) for each individual. The biological variability for nitric oxide lies within $\pm 30\%$, so individuals lying within $+30\%$ and -30% (depicted by interrupted line) can be assumed to show 'no change' in NO post step-down (43%). Individuals with $>+30\%$ change can be assumed to show a rise in NO (worsening inflammation) (36%). Those with a $<-30\%$ change demonstrated a decrease in NO (improvement in inflammation) (21%).

Figure 5: Correlation between doubling dilution shift and % change in FE_{NO}



($r = -0.14$, $P = 0.27$)

Lung function

Mean (SEM) baseline spirometry values were: FEV₁ of 86.2% (1.5), FVC 91.6% (1.4), PEF 90.2% (1.7). FEV₁ post step-down was 84.5 % (1.5), $p < 0.05$. FVC and PEF showed no significant change post step-down (91.5 % (1.4), and 90.0 % (1.6), $p = 0.87$).

AQLQ

The median (inter-quartile range) mini-AQLQ composite scores pre and post step down were 5.7 (4.7 – 6.3) vs. 6.1 (5.3 – 6.5) respectively, representing a median improvement of 0.4, $p < 0.001$.

Discussion

The present study demonstrates that supervised step down of ICS in a community setting can be achieved without any worsening of airways inflammation, as measured by FE_{NO} and mannitol challenge. This is in agreement with work carried out by Leuppi et al, who determined that the majority of patients could undergo a halving of their ICS dose without exacerbation [41]. We hypothesize that this may be due to improved adherence with supervised treatment during step down. Whilst a significant reduction in ICS could also be achieved if patients had initially been over treated, there should have been a commensurate increase in airways inflammation on withdrawal of

treatment, as has been reported elsewhere [78]. In the absence of such a change we feel that enhanced adherence is the logical conclusion. Adherence with asthma medications, particularly ICS, is known to be poor [195]. Patient-reported adherence rates are as low as 38% [196, 197], and actual drug use is often even lower. *Rand et al* compared patient-reported adherence to change in canister weight and demonstrated that although 73% of participants reported using their ICS three times daily, this was only confirmed in 15% by canister weight [197].

The commonest reasons to explain non-adherence include: patients' perception of their disease; such that they only take ICS when they become unwell as opposed to using it as 'preventer' therapy [196]; and concerns over potential side effects [195]. There is evidence to suggest that regular disease monitoring addresses these issues and leads to benefits such as improved inhaler technique and adherence [198, 199]. In this regard, patients in the present study were reviewed fortnightly and may have additionally benefited from the positive reinforcement of regular spirometry, peak flow and symptom monitoring. Whilst this high level of supervision is too intensive to be reproduced in primary care, it suggests that the widespread practice of annual review may be too infrequent to adequately educate and support our asthmatic population.

Control of airways inflammation is of critical importance in asthma, as if left uncontrolled long-term it can lead to airway remodelling, even in asymptomatic

patients [31]. Furthermore, studies in which asthma therapy was titrated to achieve suppression of surrogates of inflammation demonstrate that exacerbation rates can be reduced by up to five-fold, when compared with treatment guided by symptoms and lung function alone [37, 38]. To address this, the present study utilised bronchial challenge with mannitol and FE_{NO} as suitable surrogates of asthmatic inflammation. Bronchial challenge tests are traditionally used to diagnose the presence of AHR, and to monitor response to treatment [200]. AHR is a key feature of persistent asthma, which is considered to correlate well with underlying airways inflammation [21]. All participants in this study were required to exhibit AHR to mannitol as one of the inclusion criteria. Hence, we believe this makes an alternative diagnosis to asthma such as eosinophilic bronchitis unlikely. Nonetheless, we appreciate that AHR may vary over time, often increasing during exacerbations and decreasing during treatment with anti-inflammatory therapy [200].

Mannitol challenge is a relatively novel indirect bronchial challenge test, which is known to be reproducible, and to correlate well with other surrogate markers of airways inflammation [136, 201]. Unlike other challenge tests, it is portable, simple, and quick to perform, and therefore ideally suited for use in primary care. Moreover, mannitol is an indirect stimulus, which induces AHR by osmotically stimulating inflammatory cells to release mediators which constrict airway smooth muscle. *Fardon et al* showed that PD_{10} correlates well with PD_{15} , allowing an abbreviated challenge to be utilised without loss of sensitivity, whilst exposing the

subject to less provocation agent [202]. The overall mean improvement observed in mannitol challenge (0.46 doubling dose shift in mannitol PD₁₀) was statistically significant, but would not be considered clinically significant. Examination of change in PD₁₀ in individual patients, as depicted in Figure 3, is more revealing than the magnitude of the overall mean shift for the group as a whole. A change in mannitol PD₁₀ of +/- 1 doubling dose in a given patient is within the limits of intra-individual biological variability. Using these criteria we identified 31% of patients who had an improvement in PD₁₀ (i.e. a change > +1 doubling dose), 52 % who had no change (i.e. a change within +/- 1 doubling dose), and 17 % who had a worsening (i.e. change < - 1 doubling dose) when comparing pre vs. post step down (Fig 3). In other words it was evident that the majority of patients (i.e. 81%) either had no change in AHR or improved. A minority of patients showed a worsening of AHR, which would be expected if subjects were initially adherent with treatment. A rate of 19% is consistent with adherence rates reported in previous studies [197]. A comparison of the baseline and post step down characteristics of these three groups revealed no significant differences in terms of spirometry, FE_{NO} or ICS dose. Analysis of mannitol PD₁₀ showed that those who improved post step down had significantly greater bronchial hyper-reactivity at baseline than the other two groups. Those who worsened post-step down had significantly more bronchial hyper-reactivity at the end of step down. This correlates well with data obtained by Leuppi et al who determined that developing AHR to mannitol during dose-reduction was a good predictor of step-down failure [41]. Changes in AHR correlated very poorly with

changes in symptoms, as subjects who either “improved” or had “no change” in AHR had statistically significant, albeit clinically insignificant, worsening of their symptom scores, whereas those who had a “worsening” had no change (table 3). Individual data for those with “improved” and “worsened” AHR post-step down are included in tables 4 and 5.

FE_{NO} is an established surrogate of airways inflammation in asthma [59] and correlates closely to sputum eosinophil counts [60, 203] and mucosal eosinophilic markers [204, 205]. When used on its own as an inflammatory surrogate to titrate ICS, the results have been disappointing [76], indeed, a recent Cochrane review has determined that FE_{NO} cannot be recommended for tailoring the dose of inhaled corticosteroids in clinical practice [206]. However, its rapid response to changes in inflammatory status [71] and acute sensitivity to ICS make it helpful when used in conjunction with other measures. It has been used as an early predictor of under-treatment (predicting exacerbation with FE_{NO} and dose reduction) [78]. In this regard our cohort showed no net change in FE_{NO} during step-down. If the biological variability of FE_{NO} is assumed to be <30% [207]: 64% of subjects demonstrated no significant increase in FE_{NO} (figure 4), These results correlate well with those of Leuppi et al who found no significant increase in FE_{NO} during dose reduction or exacerbation [41]. Similarly, we found no correlation between doubling dilution shift in mannitol challenge and % change in FE_{NO} ($r = -0.14$, $P = 0.27$). We know from previous studies that 200µg BDP is sufficient to suppress FE_{NO}, even in the presence of persisting AHR,

which may explain the disconnect with AHR, and its lack of response in these circumstances [200]. However, when this observation is taken in conjunction with mannitol AHR, we believe it offers good evidence that airway inflammation was well controlled in this study, despite the relatively rapid rate at which ICS was reduced. Larger studies with a longer duration of follow-up are required to determine whether these improvements are maintained long-term.

Despite evidence to support the use of inflammatory markers in asthmatic assessment, current clinical practice dictates that ICS should be adjusted according to patient reported symptoms and spirometry [8]. In this respect, we observed a statistically significant improvement in quality of life scores, despite the reduction in ICS dose. There were no clinically significant changes in spirometry.

Treatment of asthmatic patients in primary care has been driven by results obtained from randomised controlled trials, during which treatment compliance is reinforced. These therefore, do not reflect a real life scenario. The current paper highlights the potential pit falls of extrapolating data to a primary care setting where compliance rates may be poor. Perhaps there is a need for the development of more effective strategies which support asthmatics to use established therapies correctly rather than continue a culture of escalating therapy, without frequent review and step-down if appropriate. Improvement of asthma care in the community may require an approach that includes more

effective supervision and targeted assessment to enhance adherence and reduce airways inflammation.

In conclusion we have demonstrated that achieving a significant reduction in ICS dose is possible, without any deterioration in surrogate markers of inflammation, or quality of life. This apparent disconnect between reduction in anti-inflammatory therapy and measured inflammation may reflect enhanced adherence through frequent supervision.

Critique

This is the first study to utilise mannitol challenge in a community setting, confirming that it is safe and easy to use. Furthermore, we have demonstrated that inhaled steroids can be safely stepped down in general practice without a concomitant increase in airway inflammation. Whilst these results are encouraging, we would not advocate the back-titration of inhaled steroids in all community-based patients. We recognise that this observational study has several limitations, namely to do with study design: Firstly, we were unable to follow subjects up long term to ensure that their inflammation remained suppressed. Secondly, measuring FE_{NO} pre and post step-down was only introduced once recruitment had commenced; consequently FE_{NO} measures are not available for all subjects. Thirdly, as this was an observational study there was no control group. In order to verify our suspicions that the improvements seen were due to enhanced compliance we would like to repeat the study in a prospective fashion measuring canister weight. This study suggests that concordance with medication improves in patients who are entered into a clinical trial, indicating that perhaps a more targeted approach to asthma therapy, which includes more effective supervision, alongside the use of an appropriate “inflammometer”, such as mannitol (so patients can actually see an improvement following treatment) may be beneficial. A prospective study investigating the benefits of such a treatment strategy, as well as evaluating the benefits of mannitol as a tool for titrating asthma therapy, is required.

Part b: Determining which outcomes provide sufficient assay sensitivity for detecting dose response effects on airway and systemic markers.

Study aims:

1. To compare the pharmacodynamic characteristics of Hydrofluoroalkane (HFA) and Chlorofluorocarbon (CFC) Budesonide utilising methacholine challenge as the primary endpoint in a cross over study.
2. To determine which outcomes measures provide sufficient assay sensitivity for detecting dose response.

Pharmacodynamic Comparison of Hydrofluoroalkane and Chlorofluorocarbons Budesonide

Introduction

Inhaled corticosteroids (ICS) are the mainstay of treatment in persistent asthma [8, 192]. Budesonide is one of the most commonly prescribed ICS, and has been used effectively in the long-term management of persistent asthma for many years. A suspension aerosol formulation with chlorofluorocarbon (CFC) as the propellant was previously available for delivery of budesonide via pressurized metered-dose inhaler (pMDI). In recent years CFCs have been implicated in damage to the ozone layer, and are due to be phased out in accordance with the Montreal Protocol [208]. Hydrofluoroalkane-134a (HFA) has been found to act as adequate propellant for pMDI delivery systems without the same deleterious environmental effects. Budesonide has therefore been re-formulated in suspension with HFA as the propellant. Two strengths of budesonide HFA pMDI (100µg and 200µg per actuation) have been developed. Determination of therapeutic equivalence is essential for new drug formulations. Pharmacokinetic studies have shown the two formulations to be bioequivalent [209]. The aim of this study is therefore to present pharmacodynamic data comparing HFA and CFC formulations of budesonide delivered via pMDI.

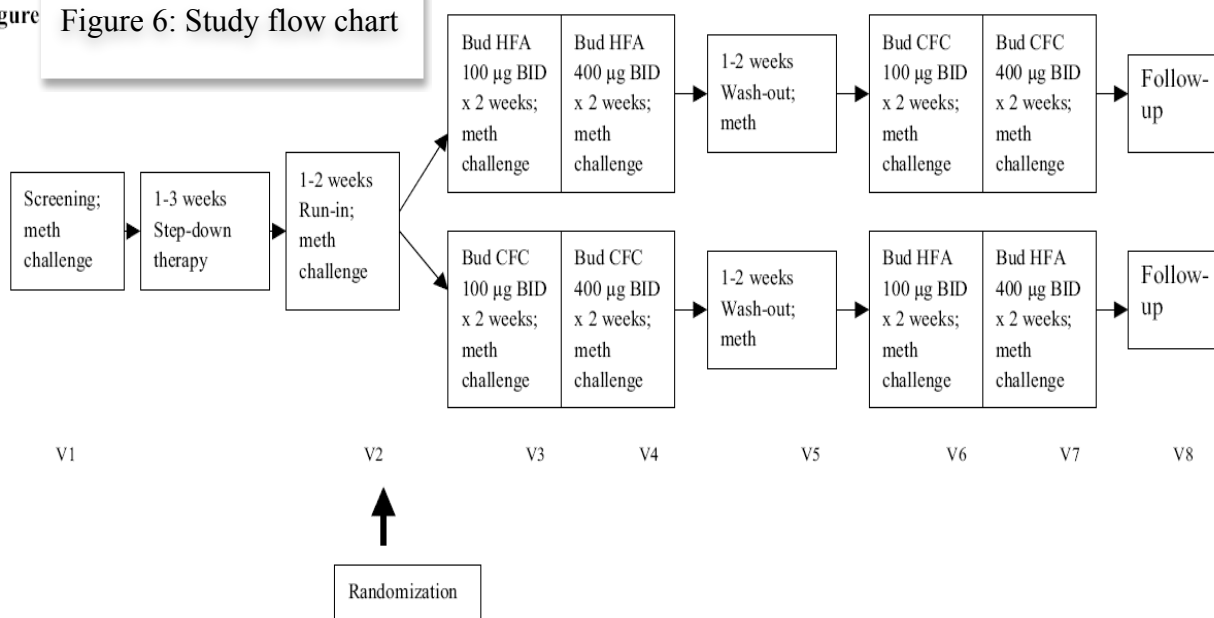
Methods

This was a single-centre, randomized, open-label, crossover study (Figure 6). At screening, entry requirements were checked and informed consent was obtained. Participants were considered eligible if they were aged 18-65 years, and had a diagnosis of stable persistent asthma while receiving $\leq 1000\mu\text{g}$ beclomethasone dipropionate (BDP) or equivalent. Subjects were excluded if they were pregnant or lactating, had concomitant respiratory disease or had been prescribed oral steroid or a change to their asthma therapy in the three months preceding the screening visit. Eligible patients then entered a step-down phase during which long-acting β_2 -agonists, theophyllines, cromones and leukotriene inhibitors were stopped. Participants then had their steroid dose gradually reduced in a manner similar to previously published studies from our department [210]. They then entered a 1-week pre-treatment (ICS-free) washout period. A methacholine PC_{20} of $\leq 4\text{mg/ml}$ and $\text{FEV}_1 \geq 60\%$ predicted post step-down was required for entry into the treatment period. Subjects not meeting the entry requirements at the end of this phase were given a further 1-2 weeks of washout.

The study consisted of two treatment periods. Subjects received low-dose ($200\mu\text{g/day}$) budesonide, either via CFC or HFA pMDI, for 2 weeks followed by a further 2 weeks of medium-dose ($800\mu\text{g/day}$) budesonide. Participants then crossed over treatment arm, effectively acting as their own control. Treatment periods were separated by a 1–2 week washout period. Subjects were required to be within 1 doubling dilution of baseline methacholine PC_{20} at the end of

washout. During all study visits participants underwent pulmonary function testing (including FEV₁, forced vital capacity, PEF, and FEF_{25-75%}), FE_{NO}, and methacholine challenge. Methacholine challenge was performed using the five-breath dosimeter technique in accordance with American Thoracic Society (ATS) recommendations [183]. The PC₂₀ (provocative dose required to cause a 20% drop in FEV₁) was calculated using log-linear interpolation of the dose response [123]. FE_{NO} was measured in line with current ATS recommendations using a NIOX analyzer (NIOX[®] Nitric Oxide Monitoring System, Aerocrine AB, Solna, Sweden) [194]. Subjects were asked to withhold short-acting β_2 -agonists and caffeine for 6 hours prior to study visits, and all study visits took place at the same time of day. At each visit inhaler technique was checked and peak flow/symptom diaries were reviewed. Subjects were asked to rate their asthma symptoms twice daily. Daytime scores were recorded each evening, and night time scores were recorded on waking. The following rating scales were used: 0 = no asthma symptoms, 1= mild (easily tolerable) symptoms, 2= moderate (interferes with normal activities/sleep) symptoms, 3= severe (prevents normal activities/sleep). Overnight (i.e. 10pm to 8am) urine was collected for cortisol and creatinine the night prior to study visits. Urinary cortisol was measured using a commercial radioimmunoassay kit (DiaSorin Ltd, Wokingham, Berkshire, UK). The intra-assay coefficient of variation was 4% and the inter-assay coefficient of variation was 8%. Urinary creatinine was measured on a Cobas-Bio auto analyzer (Roche Products, Welwyn Garden City, UK). The intra-assay and inter-assay coefficients of variation were 4.6% and 3.0% respectively.

Figure 6: Study flow chart



Statistical analysis

The primary outcome measure was change in methacholine PC₂₀ from pooled baseline (i.e. post run-in or washout periods) for each treatment. Any non-Gaussian data were log transformed prior to analysis. Gaussian data were assessed using analysis of variance (ANOVA). To compare the relative microgram potency of the two devices, Finney's bio-assay was used to estimate the relative potency. The relative potency is defined as the ratio of doses estimated to provide the same effect. Fieller's theorem allowed for the calculation of 95% CIs for the relative potency [211]. The 95% CI for relative potency was required to be contained within equivalence limits of +/-50% (i.e. a ratio of 0.5-2.0 fold). Sample size was based on the results of a previous study by Lipworth

et al [212]. A comparison was also made between the two formulations at each dose using the EMEA equivalence limits of $\pm 33\%$ (ratio of 0.67-1.50).

Other outcome variables were considered as secondary and were analyzed using a similar model but without log transformation (except FE_{NO} and OUCC).

Results

133 adults with mild-moderate asthma were screened; 99 were randomized, and 68 completed the study according to protocol. The demographic characteristics of subjects randomized to first treatment at screening are given in Table 4. Reasons for non-randomization included: $FEV_1 \leq 60\%$ predicted (n=4); $PC_{20} > 4\text{mg/ml}$ (n=13); instability in step-down (n=8); voluntary discontinuation (n=8); lost to follow-up (n=1). During the study 31 patients were withdrawn for the following reasons: failure to wash-out (n=14); worsening of asthma (n=10); personal reasons (n=4); adverse events (n=3). Both treatment sequences were well matched for age, gender, height, weight and race.

Primary efficacy variable

There were no significant differences between baseline values prior to each treatment (i.e. after each wash-out period) for any of the primary or secondary outcome measures (table 6). Therefore, all analyses were performed compared with pooled baseline values. There was also no significant difference in compliance between the two treatments, or between doses.

Table 6: Baseline characteristics prior to first treatment with HFA and CFC**pMDI**

Variable	Before HFA	Before CFC
Age (years)*	40.0 (14.13)	39.5 (14.65)
Sex (male:female)	20:24	19:26
Race (Caucasian: Black: Oriental: Other)	42:0:1:1	42:0:0:3
Methacholine PC ₂₀ (mg/ml)	0.68 (0.33 – 1.03)	0.62 (0.31 – 0.93)
FE _{NO} (ppb)	34.42 (23.62 – 45.22)	34.76 (25.13 – 44.39)
Spirometry		
FEV ₁ (L)	2.86 (2.62 – 3.10)	2.80 (2.55 – 3.05)
FVC (L)	3.83 (3.50 – 4.16)	3.75 (3.42 – 4.08)
FEF _{25–75} (L)	2.47 (2.15 – 2.79)	2.42 (2.09 – 2.75)
PEF (L/min)	445.25 (408.97 – 481.53)	438.80 (402.54 – 475.06)
Cortisol/ creatinine ratio (nmol/ mmol)	3.49 (2.40 – 4.58)	3.50 (2.35 – 4.65)

Abbreviations: FEF_{25–75}, forced expiratory flow between 25% and 75% of forced vital capacity; FEV₁, forced expiratory volume in 1 second; PC₂₀, concentration of methacholine required to produce a 20% decrease in FEV₁; PEF, peak expiratory flow.

Data are presented as geometric mean (95%CI) unless otherwise indicated. Baseline values are shown after washout before each randomized treatment period.

* Data are presented as arithmetic mean (SD).

The number of patients who had both baseline and post-treatment data measured for the primary outcome variable (methacholine PC₂₀) was n=80 for low-dose HFA, n=79 for medium-dose HFA, n=79 for low-dose CFC, and n=78 for medium-dose CFC. Significant improvements in methacholine PC₂₀ were observed following treatment with both budesonide HFA and CFC formulations. The geometric mean shift from baseline following treatment with 200µg and 800µg budesonide HFA pMDI was 1.55-fold (95%CI 1.34–1.78) and 2.16-fold (95%CI 1.88–2.50), respectively. Similar changes were observed following treatment with 200µg and 800µg budesonide CFC: 1.67-fold (95%CI 1.45–1.93) and 2.08-fold (95%CI 1.80–2.40), respectively. Both formulations demonstrated a significant dose response between 200µg and 800µg (Table 7). The geometric mean difference in PC₂₀ shift between 800ug vs. 200ug budesonide was 1.24 (95%CI 1.02–1.51; *P*=0.03) for CFC and 1.40 (95%CI 1.15–1.67; *P*=0.0008) for HFA. There were no statistically significant differences for either dose between

the two propellant types (Table 8): 200ug/day: HFA vs. CFC 0.93 (95%CI 0.76–1.13; $P=0.44$), 800ug/day: HFA vs. CFC 1.04 (95%CI 0.85–1.27; $P=0.70$). Both of these 95%CI were contained within $\pm 33\%$ equivalence limits (ratio of 0.67–1.50)

The log-linear dose-response curves for HFA and CFC were parallel and the common slope was highly statistically significant ($p<0.0001$). The estimated relative potency between HFA and CFC budesonide, for the full analysis set ($n=89$), was 1.10 (95%CI 0.49–2.66). The 95% CI was not completely contained within the $\pm 50\%$ equivalence limits of 0.5–2.0.

Secondary efficacy/safety variables

There were statistically significant differences between medium and low doses of budesonide for both CFC and HFA formulations (except morning rescue medication and pulmonary function) (Table 7). There were no statistically significant differences when comparing low-dose budesonide CFC and HFA, and medium-dose budesonide CFC and HFA (Table 8). Relative potencies were calculated for secondary outcomes that were close to unity (Table 9). Only three patients discontinued following adverse events: two lower respiratory tract infections (CFC formulation); one oral candidiasis (HFA formulation). No serious adverse events were reported in this study.

Table 7: Comparison between doses for all outcomes with each formulation

Outcome	800µg vs. 200µg HFA		800µg vs. 200µg CFC	
	Mean	95% CI (P value)	Mean	95% CI (P value)
Methacholine PC ₂₀ ratio	1.40	1.15 – 1.70 (<0.01)	1.25	1.02 – 1.51 (0.03)
FE _{NO} ratio	0.86	0.80 – 0.93 (<0.01)	0.89	0.82 – 0.96 (<0.01)
Spirometry				
FEV ₁ (L)	0.02	-0.02 – 0.07 (0.27)	0.01	-0.03 – 0.06 (0.51)
FVC (L)	0.01	-0.03 – 0.06 (0.55)	-0.01	-0.05 – 0.04 (0.78)
FEF _{25–75} (L)	0.04	-0.05 – 0.12 (0.38)	0.04	-0.04 – 0.13 (0.30)
PEF (L/min)	2.95	-5.11 – 10.99 (0.47)	-2.83	-10.93 – 5.23 (0.49)
OUCC ratio	0.67	0.54 – 0.83 (<0.01)	0.80	0.64 – 0.99 (0.04)
Diary card data*				
Morning PEF (L/min)	11.31	4.76 – 17.86 (<0.01)	9.37	2.75 – 15.99 (0.01)
Morning rescue medication (no. puffs)	-0.26	-0.41 – -0.11 (<0.01)	-0.12	-0.27 – 0.04 (0.13)
Evening rescue medication (no. puffs)	-0.24	-0.43 – -0.05 (0.01)	-0.20	-0.39 – 0.01 (0.04)
Total rescue medication (no. puffs)	-0.48	-0.81 – -0.16 (<0.01)	-0.34	-0.67 – -0.02 (0.04)
Symptoms (0–3)*				
Morning asthma symptoms	-0.11	-0.18 – -0.04 (<0.01)	-0.09	-0.16 – -0.02 (0.01)
Evening asthma symptoms	-0.15	-0.22 – -0.08 (<0.01)	-0.09	-0.17 – -0.02 (0.01)
Total asthma symptoms	-0.26	-0.39 – -0.13 (<0.01)	-0.19	-0.32 – -0.05 (<0.01)

Abbreviations: FEF_{25%–75%}, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; PEF, peak expiratory flow; OUCC overnight urine cortisol/creatinine

Data for PC₂₀, FE_{NO} and OUCC shown as the geometric mean ratio between 800 vs. 200, for other outcomes the differences between doses are given in the units specified.

Table 8: Comparison between formulations for all outcomes at each dose

Outcome	HFA 200µg vs. CFC 200µg		HFA 800µg vs. CFC 800µg	
	Mean	95% CI (P value)	Mean	95% CI (P value)
Methacholine PC ₂₀ (mg/ml)	0.92	0.76 – 1.13 (0.44)	1.04	0.85 – 1.27 (0.71)
FE _{NO} (ppb)	0.99	0.91 – 1.07 (0.78)	0.96	0.89 – 1.05 (0.38)
Spirometry				
FEV ₁ (L)	-0.02	-0.06 – 0.02 (0.34)	-0.01	-0.05 – 0.03 (0.62)
FVC (L)	-0.02	-0.06 – 0.03 (0.42)	0.00	-0.04 – 0.05 (0.97)
FEF ₂₅₋₇₅ (L)	-0.05	-0.13 – 0.04 (0.29)	-0.05	-0.14 – 0.03 (0.23)
PEF (L/min)	-2.26	-10.57 – 6.06 (0.41)	3.52	-4.87 – 11.91 (0.41)
OUC				
Cortisol/ creatinine ratio (nmol/ mmol)	1.04	0.83 – 1.31 (0.74)	0.87	0.69 – 1.10 (0.24)
Cortisol (nmol/L)	0.99	0.78 – 1.28 (0.99)	0.86	0.67 – 1.11 (0.25)
Diary card data*				
Morning PEF (L/min)	-2.19	-8.61 – 4.23 (0.50)	-0.23	-7.05 – 6.56 (0.94)
Morning rescue medication (no. puffs)	0.09	-0.06 – 0.24 (0.24)	-0.05	-0.21 – 0.11 (0.56)
Evening rescue medication (no. puffs)	0.06	-0.13 – 0.24 (0.56)	0.02	-0.18 – 0.21 (0.86)
Total rescue medication (no. puffs)	0.19	-0.13 – 0.50 (0.25)	0.05	-0.29 – 0.38 (0.78)
Symptoms (0–3)*				
Morning asthma symptoms	0.002	-0.07 – 0.07 (0.95)	-0.01	-0.08 – 0.06 (0.78)
Evening asthma symptoms	0.02	-0.05 – 0.09 (0.58)	-0.04	-0.11 – 0.03 (0.30)
Total asthma symptoms	0.02	-0.11 – 0.15 (0.74)	-0.05	-0.18 – 0.09 (0.47)

Abbreviations: CI, confidence interval; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; OUC, overnight urinary cortisol-creatinine ratio; PC₂₀, concentration of bronchial provocation agent causing a 20% decrease in forced expiratory volume in 1 second; PEF, peak expiratory flow.

Data for PC₂₀, FE_{NO} and OUC shown as the geometric mean ratio between HFA vs. CFC at either 200µg or 800µg doses. For other outcomes the differences between formulations are given in the units specified.

Table 9: Summary of relative dose potency for budesonide

Parameter	Relative dose potency	Estimate	95% Confidence interval
PC ₂₀	HFA/CFC	1.104	0.489, 2.660
Morning PEF *	HFA/CFC	1.186	0.611, 2.523
Morning asthma symptoms*	HFA/CFC	0.949	0.413, 2.117
Evening asthma symptoms*	HFA/CFC	0.913	0.481, 1.681
Total asthma symptoms*	HFA/CFC	0.929	0.483, 1.740

*From diary

95%CI for relative dose potency was determined using Fieller's theorem.

Discussion

The aim of this study was to determine whether budesonide HFA and budesonide CFC pMDI were therapeutically equivalent. Suppression of airway hyper-responsiveness (AHR) to methacholine was selected as the primary endpoint as it is a characteristic feature of asthma, which varies over time, often increasing during exacerbations and decreasing during treatment with anti-inflammatory medications [31]. AHR has been shown to correlate well with airways inflammation and to be a reliable surrogate of disease activity [34, 37]. Since asthma is an inflammatory condition and the role of ICS is to suppress inflammation, we feel that using methacholine challenge as a primary outcome is justified. The results of this study demonstrated that there was no significant difference in response between budesonide formulations in methacholine PC₂₀ with either 200ug or 800ug per day doses. The ratio of HFA to CFC was close to unity, and the 95%CI lay within +/- 33% equivalence limits (0.67-1.50) at each dose level. We also demonstrated sensitivity of the methacholine bioassay, as there was a significant dose-response relationship for both formulations. Significant dose separation has previously been reported using methacholine PC₂₀ with 100µg vs. 500µg fluticasone propionate [213]. The common slope for the overall log dose-response relationship was highly significant, demonstrating that the doses selected coincided with the steep part of the dose-response curve for BHR. The 95%CI for the relative dose potency ratio of HFA vs. CFC were close to unity at 1.10, however, the 95%CI (0.49–2.66) was outside of the +/- 50% limits of 0.5-2.0 [212]. Comparable results have been shown in a similar

dose-response study published previously from our own laboratory [212]. It could be argued that the change observed in the step up from 200µg to 800µg could be a time effect, however we feel this is extremely unlikely. Sovijarvi et al found the doubling dilution difference between fluticasone and placebo to be 1.19, 1.33 and 1.27 after 72 hours, 2 weeks and 4 weeks respectively, indicating that near maximal response in methacholine PC₂₀ is seen after 2 weeks treatment [214]. The differences observed in the present study are too large to be due to a time effect alone. FE_{NO} is another reliable surrogate of airway inflammation [59, 60, 203]. Results revealed no significant difference between products, indicating that airway inflammation was equally controlled by both formulations.

This improvement in airway inflammation was mirrored by an improvement in diary data, including symptoms and rescue medication. All measured outcomes, with the exception of spirometric measures, demonstrated a significant dose-response relationship for budesonide with both the HFA and CFC formulations. Comparison of HFA and CFC formulations at each dose demonstrated no significant differences between products for any measure. The lack of detectable dose-response for spirometric measures is likely due to our patients having mild to moderate asthma with relatively well-preserved airway calibre and little room for improvement. Previous studies have successfully demonstrated small, clinically insignificant, improvements in lung function within this time frame [200]. Spirometric indices are known to be relatively insensitive measures for assessing response to ICS in mild-to-moderate persistent asthmatic patients [147], studies

utilising these as primary endpoints have typically required large numbers of patients with prolonged follow-up [215]. Despite these issues current EMEA guidelines still recommend the use of spirometry as the primary outcome when determining the equivalent efficacy of ICS. Additionally, they recommend the use of a parallel-group study design, which requires careful matching of study groups. This raises further problems due to the inherent heterogeneity of asthma. The study design is also far more robust provided reliable, repeatable endpoints are used. The only potential concern regarding crossover studies is unequal carry-over of steroid effect between treatment periods. In this respect, for all endpoints we found no important differences between the respective pre-treatment baseline values at run-in and washout periods.

In order to be considered equivalent products also need to demonstrate similar safety profiles. HPA-axis suppression has been shown to be one of the most sensitive markers of systemic bioavailability for ICS [216]. It has also been used as a surrogate marker for potential adverse effects in other tissues. In this respect we found no significant difference between formulations at either dose. Furthermore, assay sensitivity was demonstrated in terms of a significant dose response between 200 and 800ug/day for levels of OUCC. A review of adverse events also revealed no significant differences between the two products.

Conclusion

In conclusion, pharmacodynamic assessments of HFA vs. CFC pMDI formulations of budesonide have demonstrated therapeutic equivalence in terms of airway efficacy and systemic effects. This suggests in turn that both products were therapeutically interchangeable when used in clinical practice on a puff per puff basis.

Critique

This was an extremely well designed study, which utilised a reproducible primary endpoint and a robust study design. Furthermore, it included measuring canister weight as a measure of compliance.

Chapter 4: Tailoring treatment according to clinical asthma phenotype

Part a: Smoking asthmatics

Study aims:

1. To evaluate the benefits of adding a long-acting β -2 agonist to low dose steroid vs. doubling the dose of steroid in smoking asthmatics, and compare this to non-smoking asthmatics.
2. To dissect the underlying mechanism by employing both methacholine and mannitol challenge.

Fluticasone/ Salmeterol combination confers benefits in smoking asthmatics

Introduction

Smoking is known to greatly increase the morbidity and mortality associated with asthma [149-151]. Asthmatic smokers are reported to have poorer symptom control [150] an accelerated decline in lung function over time [149], and more emergency department visits and hospitalizations than non-smoking asthmatics [151]. The reasons for this are two-fold: firstly smoking has been associated with increased airway inflammation and airway hyper-reactivity (AHR) [153, 156]. Secondly, smokers have been found to develop relative resistance to the beneficial effects of corticosteroids [157, 158, 160]. Whilst smoking cessation remains the single most effective intervention in this groups of asthmatics [217], many do not wish to consider this. Indeed, the prevalence of active smoking among asthmatics is the same as in the population at large, with rates ranging from 17-35% [218]. Alternative treatment strategies therefore need to be found.

Tomlinson et al reported that treatment with high doses of inhaled corticosteroid (2000 μ g beclomethasone) reduced the disparity of response between smokers and non-smokers with asthma [159]. However, at high doses the risk of developing long term adverse effects from inhaled corticosteroid treatment (ICS) is increased [13]. Additional controller therapy is therefore required as add on to inhaled corticosteroid. In non-smoking asthmatics the addition of long-acting

beta-2 agonists (LABAs) have been shown to have a 'steroid sparing effect', and current guidelines recommend the addition of these if control is not achieved with 200-800 μ g BDP.

A recent subgroup analysis of the GOAL study [163] has suggested that smokers gained more benefit from fluticasone/salmeterol (FPSM) vs. a higher dose of FP in terms of reduction in exacerbation rates over 12 months. Expressing the magnitude of gains in a clinical context; for non-smokers it would take 25 years to prevent an exacerbation by taking fluticasone/ salmeterol (FPSM) vs. fluticasone (FP) alone, as compared to 6.7 years in smokers [164]. The greater benefit of FPSM in smokers was thought to be due to either relative resistance to the FP moiety on asthmatic inflammation, or greater benefit of the SM moiety on airway smooth muscle.

The aim of the present study was to evaluate the effects on AHR of adding SM to FP or doubling the dose of FP, comparing smoking and non-smoking asthmatics. To further dissect the underlying mechanisms we employed two different bronchial challenges, methacholine which is a direct cholinergic smooth muscle stimulus, and mannitol that acts indirectly as an osmotic stimulus to release inflammatory mediators.

Methods

Participants

38 mild to moderate persistent stable asthmatics (17 smokers and 21 non smokers) which met the following inclusion criteria were recruited: 18-65 years, stable persistent asthma with an $FEV_1 \geq 60\%$ ($<30\%$ PEF variability) on $\leq 1000\mu\text{g}$ beclomethasone dipropionate (BDP) or equivalent, and a methacholine $PC_{20} < 4\text{mg/ml}$ at the end of run-in. Smoking asthmatics were required to have an FEV_1 / FVC ratio of $>70\%$. Those with a ratio $<70\%$ had to have a clear clinical history of asthma (diurnal variation in symptoms, symptoms in relation to exercise or allergen exposure, and lack of productive cough), and demonstrate either bronchodilator reversibility or diurnal variation in peak flow of $>15\%$ during the run-in period. Subjects with an exacerbation of asthma requiring oral steroids, a change in their asthma medications or hospitalisation in the preceding three months, or a respiratory tract infection in the preceding two months were excluded from the trial. Non-smokers were required to have never smoked or to have quit > 1 year previously. Smokers were given smoking cessation advice prior to being recruited for the trial. Only smokers who did not wish to stop smoking were recruited. They were requested to smoke a constant amount for the duration of the trial.

Study design

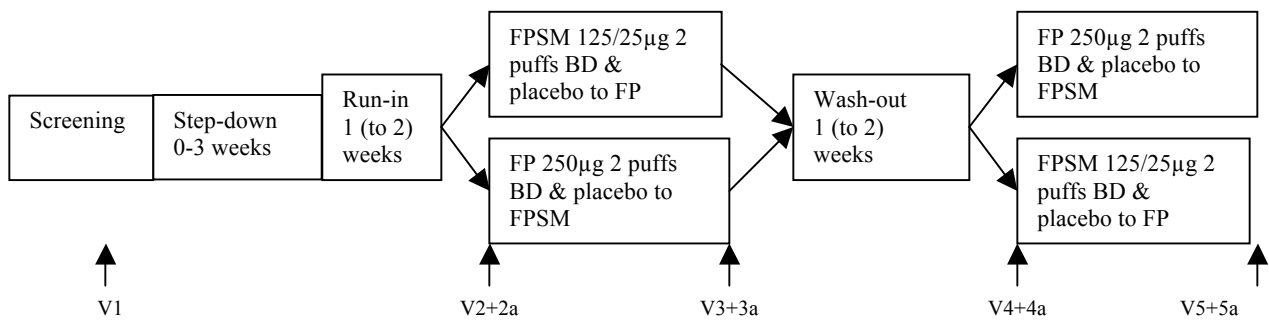
A single centre, randomised, double-blind, double-dummy, cross-over design was employed (see figure 6). The Tayside committee for medical research ethics

approved the study protocol, and all participants gave written informed consent. (eudraCT number 2008-001027-59, ClinicalTrials.gov Identifier NCT00830505). Subjects were required to stop all antihistamines, leukotriene receptor antagonists, and theophyllines for the duration of the study. Following an initial screening visit, all participants had their inhaled steroid dose halved at weekly intervals. They then entered a 1-2 week steroid free run-in period. Subjects who achieved a methacholine PC₂₀ <4mg/ml, whilst remaining clinically stable, at the end of this period (baseline) were randomised to receive either FPSM (Cipla, Mumbai, India) 125/25µg pMDI two puffs BiD and placebo to FP (Neolab, UK) or active FP (Allen and Hanbury's, Middlesex, UK) 250µg pMDI two puffs BiD and placebo to FPSM (Neolab, UK) for two weeks each. Both medications were administered through spacer devices (FP and placebo to FP via Volumatic® (GSK UK), FPSM and placebo to FPSM via Synchro-breathe® (Neolab, UK). In this regard we have previously shown that the fine particle dose and lung bioavailability of FPSM are equivalent in vitro and in vivo for Neolab and GSK formulations when used via pMDI alone or with spacer [209] while the Synchro-breathe and Volumatic produce a similar lung bioavailability for both moieties when used with FPSM via pMDI [219].

Each treatment period comprised of four study visits (see figure 7), which all took place at 2pm. On the first visit subjects underwent FE_{NO} measurement, spirometry, impulse oscillometry (IOS), methacholine challenge and blood was taken for eosinophil count, and ECP. During visit 2, which was scheduled to take

place within 1-3 days of visit 1, subjects underwent mannitol challenge. Participants were required to carry out an overnight urine collection (10 hours) prior to attending for visit 2. Subjects were issued with their study inhalers at the end of visit 2. Visit 3 was scheduled to be within 2 weeks of visit 2, and was identical to visit 1. Subjects continued their study medications until after their mannitol challenge at visit 4. Patients were required to keep peak flow diaries throughout the duration of the trial. Juniper Asthma Control Questionnaires (ACQ) was assessed at visits one and three. The treatment periods were separated by a 1-2 week steroid-free washout period. Subjects were required to be within 1 doubling dilution of their initial baseline methacholine PC₂₀ to obviate any carry-over effect.

Figure 7: Study diagram



Methacholine challenge, IOS, FeNO, Peak flow diary, spirometry, and eosinophils taken at V2, V3, V4, V5
Mannitol challenge performed and urine for OUCC collected at V2a, V3a, V4a, V5a

Measurements

FE_{NO} was recorded prior to any pulmonary function measurements using a Niox Nitric oxide analyser (Aerocrine 2 AB, Sweden) using ATS criteria [194]. Bronchial provocation testing using methacholine was performed using the five breath dosimeter technique in accordance with ATS recommendations to determine the PC₂₀ threshold at baseline, after each treatment period, and after a corticosteroid free washout using log-linear interpolation [183]. Mannitol challenge was performed by administering spray-dry mannitol powder in gelatine capsule form (Aridol™ Pharmaxis Ltd, Sydney, Australia), inhaled from a dry powder device (Osmohaler, Pharmaxis Ltd. French's Forest, NSW, Australia) as previously described by Anderson and colleagues [136]. A Jaeger Masterscreen (Erich Jaeger, Hoechberg, Germany) was used to measure impulse oscillometry according to the guidelines [188]. Subjects supported their cheeks to reduce shunting, whilst impulses were applied for 30secs during tidal breathing. All manoeuvres were performed in triplicate and means taken. Adrenal suppression from systemic absorption of the inhaled corticosteroid treatment was assessed by measuring overnight 10h urinary free cortisol and creatinine ratios as described previously in literature [220].

Laboratory assays

All assays were performed in duplicate in a blinded fashion. The serum ECP was measured using an enzyme linked immunoassay technique (UniCAP; Sweden Diagnostics UK Ltd, Milton Keynes, UK) with an intra-assay co-efficient of variation of 3.3%. Blood Eosinophil count was analysed using the Sysmex XE 2100 Hematology auto analyzer. The urinary cortisol was measured using a commercial radioimmunoassay kit (DiaSorin Ltd, Wokingham, Berkshire, UK), which has no cross reactivity with fluticasone. The intra assay coefficient of variation was 4% and the inter assay coefficient of variation was 8%. Urinary creatinine was measured on a Cobas-Bio auto analyser (Roche Products, Welwyn Garden City, UK). The intra assay and inter assay co-efficient of variation was 4.6% and 3% respectively.

Statistical analysis

SPSS version 16 (SPSS Inc, Chicago, IL, USA) was used to carry out the statistical analysis. A sample size of 13 completed patients was estimated to give >80% power to detect a one doubling dilution (dd) difference (minimal important difference) in methacholine PC₂₀. The sample size estimations were supported by a previous studies from our department where the within subject standard deviation was 0.95dd [221]. Data were analysed within each group for patients who completed the crossover study per protocol. Any non-Gaussian data were log transformed prior to analysis. Gaussian data were assessed using paired and unpaired t-tests. Post run-in and washout were compared with a paired t-test to

demonstrate no carry over effect. The primary outcome measure was change in methacholine PC₂₀ from respective baseline (i.e. post run-in or washout periods) for each treatment. All other outcomes were considered secondary. Response-dose-ratios and ACQ were subjected to non-parametric analysis. Median differences were calculated within group using the Wilcoxon Rank-Sum test for paired data, and between group using the Mann-Whitney test.

Results

38 participants were randomised, of which 31 completed per protocol (16 non-smokers, 15 smokers). 5 withdrew because of personal reasons, 2 had an FEV₁ <60% following wash-out. The mean (SEM) cotinine levels for non-smokers was 4.37 (1.26) ng/ml, while all smokers had cotinine levels above 50 ng/ml. Baseline data for smokers and non-smokers are shown in table 10. 13/15 smokers had no baseline obstruction (i.e. FEV₁/FVC ratio of >70%), the 3/15 who did not demonstrated >15% variability in PEF_R. There was no significant difference in pre-treatment baseline vs. post washout values for the primary outcome. Similarly, there were no differences in pre-treatment baselines for all secondary outcomes, except FE_{NO}.

Table 10: Baseline characteristics

Parameter	Non smokers	Smokers	P value
Methacholine PC ₂₀ (mg/ml)	0.76 (0.34 – 1.69)	1.02 (0.58 – 1.78)	0.32
Methacholine RDR (%/ml) †	6.05 (3.67- 46.80)	9.07 (3.39 – 17.81)	0.86
Mannitol PD ₁₅ (mg)	158.8 (56.7 – 444.8)	123.6 (67.9 – 225.3)	0.68
Mannitol RDR (%/mg) †	0.04 (0.03 - 0.12)	0.06 (0.04 - 0.21)	0.17
Spirometry (% predicted)*			
FEV ₁	82.7 (2.9)	87.9 (3.1)	0.24
FVC	98.4 (2.3)	99.4 (3.0)	0.49
PEF	92.6 (4.4)	98.4 (4.1)	0.58
FEF ₂₅₋₇₅	71.2 (2.4)	75.4 (2.4)	0.66
FE _{NO} (ppb)	41.2 (26.4 – 64.4)	13.8 (8.9 – 21.4)	0.04
Impulse oscillometry (kPa.L ⁻¹ .s)*			
R5	0.47 (0.08)	0.55 (0.14)	0.05
R20	0.37 (0.06)	0.44 (0.10)	0.03
R5-20	0.09 (0.02)	0.12 (0.02)	0.04
X5	-0.14 (0.02)	-0.18 (0.02)	0.20
AM peak flow (l/min)*	441 (23.3)	384 (22.6)	0.09
PM peak flow (l/min)*	447 (21.5)	394 (24.1)	0.11
ACQ†	1.1 (0.0-2.0)	1.9 (0.4-3.0)	0.03
Reliever use (puffs/day) †	1.5 (0-5)	2.4 (0-5)	0.07

Data presented as geometric mean (95% confidence intervals) unless otherwise stated

* Data presented as mean (SEM)

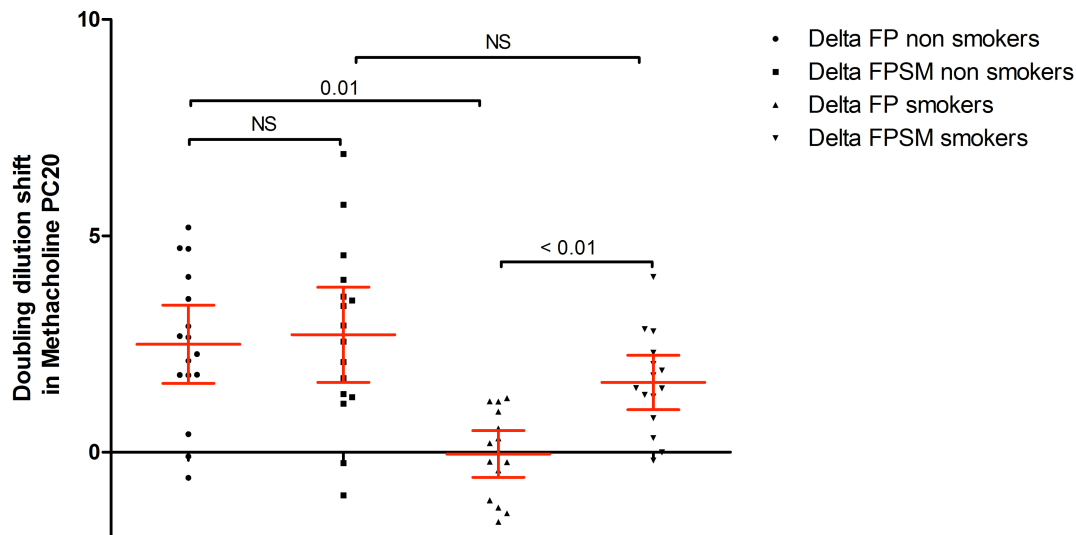
†Data presented as median (range)

Primary outcome

Both treatments led to similar significant improvements in methacholine PC₂₀ from pre-treatment baseline in non-smokers (see figure 8): FP produced a 2.34dd shift (95%CI 1.43–3.26, P<0.01); FPSM produced a 2.72dd shift (95%CI 1.54–3.91, P<0.01). There was no significant difference between treatments: 0.22dd (95%CI -1.03–1.47) P=0.71. Smokers did not gain any benefit from FP as change from baseline: -0.04dd (-0.58–0.58) P=0.88. However, in smokers there was a 1.62dd (95%CI 0.99–2.24) P<0.01 improvement with FPSM. This equated to a 1.65dd (95%CI 0.92–2.39) difference between FPSM and FP, P<0.01. There was a significant difference in response to FP between smokers and non-smokers: 2.54dd (95%CI 1.51–3.56) P<0.01, but not in response to FPSM: 1.10dd (95%CI -0.13–2.34), P=0.08. When comparing smokers vs. non-smokers, there was a greater benefit from FPSM vs. FP 1.4dd (95%CI 0.01–2.8), P<0.05.

Methacholine response-dose-ratio (RDR) (% max fall in FEV1/cumulative dose) showed similar trends: non-smokers demonstrated median (inter-quartile range) 4.69 (1.56–45.50, $P<0.01$) vs. 5.56 (3.02 – 50.08, $P<0.01$) %/ml improvements from baseline with FP and FPSM respectively. There was no significant difference between treatments (i.e. delta-FP vs. delta-FPSM), $P=0.84$. Smokers demonstrated 0.63%/ml (-4.30–5.46, $P=0.80$) vs. 5.44 %/ml (2.51–16.84, $P<0.01$) improvements from respective baseline with FP and FPSM respectively. There was a significant ($P=0.02$) difference between treatments (delta-FP vs. delta-FPSM).

Figure 8: Change in methacholine PC_{20} from respective baseline



Data presented as geometric mean (95% confidence intervals)

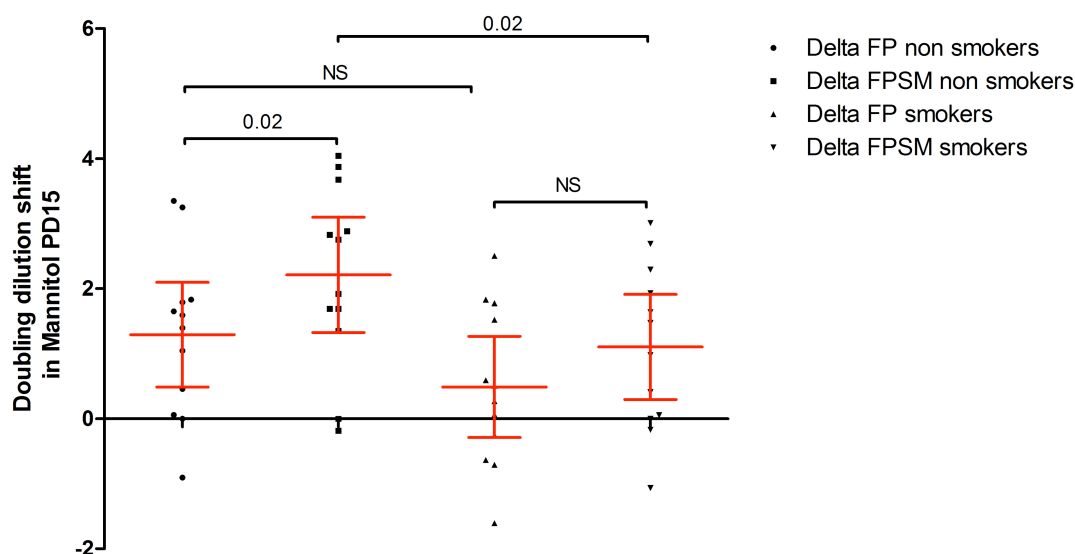
Secondary outcomes

Mannitol

There were more mannitol non-responders amongst the non-smokers than smokers: 5/16 (31%) vs. 2/15 (13%). Non-responders at either baseline were therefore removed and not included in the per protocol analysis of PD₁₅. In total 11 non-smokers and 12 smokers were included in the per protocol analysis. Non-smokers gained significant benefit from both FP and FPSM: 1.41 dd (95%CI 0.57–2.26), $P<0.01$, and 2.41dd (95%CI 1.56–3.26), $P<0.01$ respectively. FPSM conferred a significantly greater improvement in mannitol PD₁₅ in non-smokers than FP: a difference of 1.00dd (95%CI 0.23–1.78), $P=0.02$. Smokers had no significant improvement following treatment with FP: 0.59dd (95%CI -0.23–1.42), $P=0.24$, but FPSM caused a significant improvement: 1.20dd (0.34–2.07), $P=0.02$. There was a significant difference in response to FPSM between non-smokers vs. smokers: 1.31dd (95%CI 0.21–2.41), $P=0.02$, but not in response to FP: 0.92dd (95%CI -0.15–2.00), $P=0.09$. Analysis of the RDR for mannitol revealed similar trends to PD₁₅, but allowed the inclusion of all 31 cases: Non-smokers demonstrated significant improvement from respective baseline with both treatments: median (inter-quartile range) 0.02 %/mg (0.003–0.07, $P<0.01$) and 0.04 %/ml (0.01–0.15, $P<0.01$) with FP and FPSM respectively. Smokers demonstrated significant improvement from respective baseline with FPSM: 0.02 (0.01–0.80), $P=0.03$, but not with FP: 0.01 (-0.02–0.09), $P=0.25$. There were no

significant differences between treatments for either FP ($P=0.22$) or FPSM ($P=0.51$).

Figure 9: Change in mannitol PD15 from respective baseline



Data shown as geometric mean (95% confidence interval)

Table 11: Change from respective baseline in challenge outcomes

		Change from respective baseline with FP	Change from respective baseline with FPSM	(FPSM vs. FP) P value
Methacholine	S	-0.04 (-0.58 – 0.58)	1.62 (0.99 – 2.24)*	<0.01
(dd shift)	NS	2.50 (1.43 – 3.26)*	2.72 (1.54– 3.91)*	0.71
Difference in MCT	S	0.63 (-4.30 – 5.46)	5.44 (2.51 – 16.8)*	0.02
RDR (%/ml) †	NS	4.69 (1.56 – 45.50)*	5.56 (3.02 – 50.1)*	0.84
Mannitol	S	0.59 (-0.23 – 1.42)	1.20 (0.34 – 2.07)*	0.17
(dd shift)	NS	1.41 (0.57 – 2.26)*	2.41 (1.56 – 3.26)*	0.02
Difference in	S	0.01 (-0.02 – 0.09)	0.02 (0.01 – 0.08)*	0.76
Mannitol RDR	NS	0.02 (0.003 – 0.07)*	0.04 (0.01 – 0.15)*	0.17
(%/mg) †				

RDR= response-dose-ratio (% max fall in FEV1/cumulative dose), dd= doubling dilution

All data presented as geometric mean (95% confidence interval)

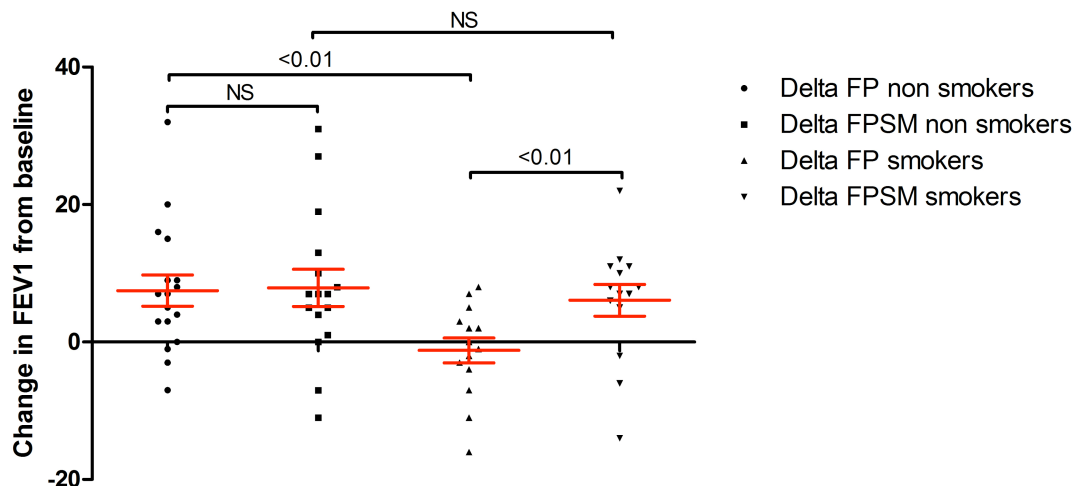
* significant change from respective baseline

†Data presented as median (range)

Measures of airway calibre

Non-smokers saw a significant improvement in mean (SEM) FEV₁ (% predicted) with both treatments: 5.94% (1.78), $P < 0.01$ and 7.88% (2.72), $P = 0.01$ with FP and FPSM respectively. There were no significant differences between treatments ($P = 0.57$). Smokers saw significant improvement with FPSM but not with FP: 6.07% (2.32), $P = 0.02$ and -1.21% (1.80), $P = 0.51$ respectively. Non-smokers gained significantly greater benefit from FP than smokers, 8.68% (2.99), $P < 0.01$, but there was no difference between groups for FPSM, -1.66% (5.20), $P = 0.62$. Differences in FVC, PEF, FEV₂₅₋₇₅, IOS, and morning peak flow are detailed in table 12.

Figure 10: Change in FEV1 from respective baseline



Data shown as mean (SEM)

Table 12: change from respective baseline with measures of airway calibre

		Change from respective baseline with FP	Change from respective baseline with FPSM	Mean difference	P value
Spirometry (% predicted)					
FEV ₁	S	-1.21 (1.80)	6.07 (2.31)*	7.29 (2.24 – 12.32)	<0.01
	NS	5.94 (1.78)*	7.88 (2.72)*	1.94 (-5.08 – 8.96)	0.57
FVC	S	2.79 (1.79)	-2.36 (2.23)	-5.14 (-10.87 – 0.59)	0.07
	NS	4.13 (1.85)*	3.13 (1.85)	-1.00 (-6.59 – 4.59)	0.71
PEF	S	0.71 (2.35)	8.14 (2.74)*	7.43 (1.92 – 15.79)	0.02
	NS	3.44 (1.28)*	8.50 (2.78)*	5.06 (-0.84 – 10.96)	0.09
FEF ₂₅₋₇₅	S	2.01 (2.76)	9.44(3.03)*	7.44 (-2.52 – 17.39)	0.13
	NS	9.84 (3.98)*	14.1 (4.61)*	4.25 (-2.55 – 11.05)	0.12
Impulse oscillometry (kPa.L ⁻¹ .s)					
R5	S	0.01 (0.03)	0.08 (0.02)*	0.07 (0.002 – 0.17)	0.06
	NS	0.07 (0.02)*	0.06 (0.02)*	-0.01 (-0.07 – 0.08)	0.85
R20	S	-0.003 (0.02)	0.03 (0.02)*	0.04 (-0.02 – 0.10)	0.20
	NS	0.03 (0.02)	0.02 (0.01)	-0.01 (-0.03 – 0.05)	0.67
X5	S	0.01 (0.02)	0.03(0.01)*	0.02 (-0.02 – 0.07)	0.31
	NS	0.03 (0.01)*	0.04(0.01)*	0.003 (-0.03 – 0.04)	0.86
R5-20	S	-0.01 (0.01)	0.04 (0.01)*	0.05 (0.01 – 0.08)	0.03
	NS	0.04 (0.03)	0.04 (0.01)	0.005 (-0.08 – 0.09)	0.90
Am PEFR (l/min)	S	6.1 (6.7)	4.9 (4.5)	-1.0 (7.6)	0.90
	NS	33.9 (6.7)*	20.2 (6.1)*	-13.8 (8.7)	0.14
Pm PEFR (l/min)	S	9.5 (6.3)	1.4 (2.8)	-8.2 (1.5)	0.27
	NS	30.3 (8.1)*	23.9 (7.4)*	-6.4 (1.1)	0.50

PEFR= change in average peak flow rate (over 1 week preceding the study visit) from respective baseline

R5= total airway resistance, R20= proximal airway resistance, X5= peripheral reactance, R5-20= peripheral airway resistance.

Mean diff represents the magnitude of change between each treatment

*Significant change from respective baseline

All data presented as mean (SEM)

FE_{NO}

Baseline FE_{NO} was significantly lower in smokers than non-smokers: 13.8ppb (95%CI 8.9-21.4) and 41.2ppb (95%CI 26.4–64.4) respectively, P=0.04. Non-smokers showed significant reductions from baseline in FE_{NO} with both treatments: 55% (95%CI 37.4–68.3), P<0.01 with FP and 47% (95%CI 27.5–61.8) P=0.02 with FPSM. There were no significant differences between treatments, 19% (95%CI -10-40), P= 0.16. Smokers demonstrated a 4% (95%CI

-33.3–37.1) reduction in FE_{NO} post FP ($P=0.49$), and a 47% (95%CI 34.1–57.5) reduction post FPSM ($P<0.01$). However, the pre-FPSM baseline in smokers was significantly higher than the pre-FP baseline: 13.7ppb (95%CI 8.9–21.4) $P=0.04$. There was a 54% (25–71) difference between the response to FP in non-smokers vs. smokers, $P<0.01$, but no significant difference for response to FPSM, 0.3% (95%CI -31–44), $P=0.99$.

ACQ

Non-smokers had significantly lower scores at baseline than smokers: median (range) 1.1 (0.0–2.0) and 1.9 (0.4–3.0) respectively. Non-smokers showed significant improvements in ACQ with both FP ($P=0.01$) and FPSM ($P=0.02$). There was no significant difference between treatments ($P=0.57$). No improvements were seen in smokers with either treatment: $P=0.31$ and $P=0.32$.

Laboratory measures

Neither treatment caused significant suppression of blood eosinophil counts from baseline: -0.01 (95%CI -0.05–0.04), $P=0.13$ vs. 0.03 (95%CI -0.03–0.09), $P=0.53$ in non-smokers, and 0.01 (-0.07–0.09), $P=0.61$ vs. 0.04 (-0.01–0.09), $P=0.71$ in smokers, with FP and FPSM respectively. In keeping with the eosinophil data, neither treatment led to a significant reduction in ECP from respective baselines: -0.04 (95%CI -0.18–0.11), $P=0.60$ vs. 0.05 (95%CI -0.07–0.17), $P=0.41$ in non-smokers, and -0.04 (95%CI -0.16–0.08), $P=0.51$ vs. 0.04 (95%CI -0.13–0.21), $P=0.63$ in smokers, with FP and FPSM respectively. In smokers FP produced

significant adrenal suppression of OUCC compared to baseline: -2.82 (1.24) nmol/mmol ($P=0.03$), but not with FPSM, -0.90 (0.91) nmol/mmol, $P=0.34$. In non-smokers neither treatment produced a significant fall in OUCC: -1.24 (1.01) nmol/mmol ($P=0.24$) and 0.12 (0.74) nmol/mmol ($P=0.88$) with FP and FPSM respectively.

Discussion

Studies in non-smokers have shown LABA's to have a relative 'steroid sparing effect', allowing comparative improvements in lung function and reductions in exacerbations to doubling the dose of inhaled steroid [221-223]. This is in keeping with our non-smoking cohort, which demonstrated significant improvements in AHR, FE_{NO} , and spirometric indices that were comparable between treatments. Smokers on the other hand, showed significantly greater improvements from the addition of SM to FP (500ug/day) compared to doubling the dose of FP alone (1000ug/day), in terms of AHR (methacholine PC_{20} and RDR), spirometry (FEV1) and IOS (R5-R20). Furthermore, smokers appeared to gain proportionally greater benefit from the addition of SM than non-smokers on methacholine AHR. These results support the observation that smokers would need 18 years less treatment, by taking FP/SM vs. FP alone, to prevent an exacerbation than non-smokers [164].

Current asthma guidelines emphasize the importance of establishing asthmatics on ICS prior to addition of long-acting b2-agonist [8]. The OPTIMA trial clearly demonstrated that the addition of low dose ICS in steroid naïve individuals led to

a significant reduction in severe exacerbations and improvements in asthma control. Whereas the addition of a LABA led to improvements in lung function, but did not confer any additional clinical benefit [192]. In the present study we found no significant benefit, in any primary or secondary outcome, from high dose inhaled FP (1000ug/day) in smokers. Whilst smoking asthmatics are known to develop relative steroid resistance [157], it is currently thought that this can be partially overcome through increased doses [159]. This assumption is based on a study by Tomlinson et al, who demonstrated that high dose ICS decreased peak flow variability to a similar degree in both smokers and non-smokers [159]. Peak flow, like spirometry, reflects airway caliber and as such is relatively distant from the underlying asthmatic inflammatory process [21]. In this respect we saw no significant improvement in either peak flow dairy cards or ACQ in smokers. It could be argued that two weeks duration of treatment with FP may not have been sufficient to observe the changes in PEF seen by Tomlinson et al, however, it has previously been shown by Sovijarvi et al that 2 weeks is sufficient in achieving near maximal effects on AHR in response to FP 250ug bid via spacer [214].

Our result has important implications as it suggests that higher doses of ICS fail to control inflammation in smokers, exposing them to significant risk of side effects. Addition of LABA on the other hand, allows improvements in AHR similar to those seen in non-smokers. We showed a significant systemic effect on adrenal suppression in smokers with FP 500µg bid but not with FPSM 250µg bid. This further emphasizes the importance of adding salmeterol rather than

increasing the dose of FP, in terms of optimizing the risk-benefit. Whilst smoking cessation should always be advocated as the best and most effective treatment for persistent asthma [217], we would advocate that smoking asthmatics who do not wish to consider cessation be started on combination therapy as first line rather than being treated with inhaled steroid alone.

Some mechanistic insights can also be gleaned by examining the relative effects on mannitol and methacholine challenges. Mannitol is an indirect osmotic challenge, which is thought to correlate more closely with airway inflammation than methacholine, a direct bronchial smooth challenge [90]. A study utilising AMP, another indirect challenge, found FPSM 250µg bid to be inferior to 500µg bid in non smokers [221]. Since mannitol has been shown to correlate closely with AMP challenge [201], the results obtained in the present study were somewhat surprising in that improvements in mannitol PD₁₅ following FPSM were greater than FP in non-smokers. Since SM exhibits no clinically meaningful *in vivo* anti-inflammatory activity [224], the superiority of FP/SM vs. FP alone on mannitol in non-smokers is likely due to an alternative mechanism: LABAs are known to have a stabilizing effect on airway smooth muscle [225]; to increase mast cell stabilization, thereby inhibiting the release of inflammatory mediators such as histamine or cysteinyl leukotrienes [226] and to preserve the integrity of the respiratory epithelium [227]. This in turn suggests that mannitol may be less specific for inflammation than originally thought. However, we did not see any difference in effect on methacholine AHR in non-smokers between FPSM vs. FP,

that is perhaps, counter intuitive since methacholine acts directly on airway smooth muscle.

In smokers we saw a significant difference between FPSM vs. FP on methacholine AHR, with a similar non-significant trend on mannitol. The greater improvements with FP/SM in smokers were also seen on airway caliber on outcomes of spirometry such as FEV1 and on impulse oscillometry on R5-R20. The effect of salmeterol is due to a direct action on the airway smooth muscle leading to both bronchodilation and a protective effect due to functional antagonism against bronchoconstriction. This functional antagonism can be considered to be a surrogate for stabilization of airway smooth muscle and determines the degree of protection when exposed to a challenge stimulus. It therefore appears that in the face of relative steroid resistance the smooth muscle stabilization conferred by the LABA becomes relatively more important in smokers than in non-smokers. Studies looking at the effect of smoking cessation on AHR to AMP and methacholine have shown significant improvements in AMP but not methacholine 6 months after smoking cessation in quitters [226], suggesting that smokers may have a greater degree of smooth muscle dysfunction than non-smokers.

CT studies have shown smokers to have a reduced airway lumen area compared with non-smokers [228]. Similarly our results have shown that smokers have higher peripheral resistance (R5-20) at baseline compared with non-smokers, suggesting a greater degree of peripheral smooth muscle dysfunction, and

potentially increased peripheral inflammation. In combination products the bronchodilator component might conceivably result in improved peripheral lung deposition of the ICS, thereby producing enhanced anti-inflammatory activity in smaller airways. It has been suggested that oscillometry is a sensitive measure of small airway dysfunction and changes in R5-20, and X5 may reflect small airway physiology [228]. Thus, the changes in IOS demonstrated in this study with smokers may indicate beneficial small airway effects with FP/SM. The mechanism by which smoking causes FE_{NO} reduction is not fully understood, but may include reduction in NO synthesis due to feedback inhibition induced by high concentrations of NO contained in cigarette smoke [229]. Pre-treatment baselines for FP/SM vs. FP were significantly different, accounting for increased suppression with FP/SM in smokers.

In conclusion, combination therapy with FP/SM conferred significant improvements in AHR and airway caliber in smoking asthmatics as compared to double the dose of FP alone. It is likely that in the face of the relative steroid resistance seen in smokers, the bronchodilation and smooth muscle/ mast cell stabilization conferred by the LABA become more important in terms of achieving superior clinical control. Future guidelines should take into account the differences in treatment response between smokers and non-smokers.

Critique

Therapeutic studies, which form the basis for current asthma guidelines, often exclude current smokers due to concerns about recruiting participants with chronic obstructive pulmonary disease (COPD). 87% of participants in the present study had an FEV₁/FVC ratio of $\geq 70\%$ suggesting that they had no baseline obstruction, making a diagnosis of COPD unlikely. However, due to the difficulties that we encountered in recruiting smoking asthmatics, we also had to include subjects with a ratio of $< 70\%$. These subjects were required to demonstrate either $> 15\%$ bronchodilator reversibility or $> 15\%$ diurnal variation in peak flow, alongside a clinical history suggestive of asthma (i.e. diurnal variation, nocturnal, or exercise related symptoms). However, a recent study by Calverley et al has clearly demonstrated that bronchodilator response is a poor diagnostic test for COPD, as up to 52% of patients changed responder status between visits [230]. Fortunately, all 3 patients with a ratio $< 70\%$ demonstrated $> 15\%$ diurnal variation in peak flow during the run-in period. However, we acknowledge that despite the above inclusion criteria, we may have included subjects with very mild COPD. A further criticism that could be leveled at this study is that we did not include a measure of compliance.

Part b: Elite swimmers

Study aims:

1. Determine the prevalence of EIB and rhinoconjunctivitis in adolescent elite swimmers.
2. To determine what happens to airway inflammation following exposure to chlorine and exercise.
3. To compare standardised field-based exercise challenge to mannitol challenge,

Effects of chlorine and exercise on the unified airway in adolescent elite Scottish swimmers

Introduction

Elite swimmers have higher rates of rhinoconjunctivitis and exercise induced bronchospasm (EIB) compared with any other groups of athletes [168, 231]. Proposed mechanisms include a combination of chronic exposure to toxic chlorine metabolites, and high ventilatory rates leading to osmotic degranulation of mast cells and subsequent bronchoconstriction [232]. In adult elite swimmers, significantly higher levels of airway inflammatory cells have been demonstrated compared with healthy controls. This reverts back to normal following long-term cessation of the sport [172]. There is a paucity of data on the prevalence of rhinoconjunctivitis and exercise induced bronchoconstriction in adolescent elite swimmers. This is of particular importance as rhinoconjunctivitis has been shown to be an independent risk factor for developing asthma [233]. Indeed rhinitis and asthma are considered to be part of the same disease continuum, the 'unified airway' [234]. We therefore conducted a pilot study to assess the combined effects of chlorine and exercise on the unified airway of adolescent elite swimmers, including estimating the prevalence of rhinoconjunctivitis and EIB.

Methods

Participants and study design

The Scottish Midland District swimming squad (36 swimmers) were assessed during a 2 hour training session. All swimmers underwent exhaled tidal (FE_{NO}) and nasal (N_{NO}) NO measurement peak nasal inspiratory flow rate (PNIF), and FEV_1 before and after swimming. A sport-specific exercise test was carried out during an intensive aerobic set. All swimmers completed a health questionnaire. Swimmers were asked to withhold anti-histamines, leukotriene receptor antagonists (LRTAs), nasal steroid sprays, and long acting β_2 - agonists (LABAs) for 1 week before the training session. Inhaled corticosteroid inhalers could be taken as normal. Participants were asked to refrain from taking short acting β_2 -agonists (SABAs) 12 hours before the session. This study was approved by the local ethics committee (REC ref: 09/S1402/6) and all participants gave written informed consent.

Measurements

NO measurement

All participants underwent measurement of FE_{NO} using a portable MINO (NIOX MINO® Airway Inflammation Monitor; Aerocrine AB, Solna, Sweden). A single reading was obtained in accordance with manufacturer's guidelines. Nasal exhaled NO was measured using an adapted MINO device, as previously described [235].

Sport-specific field-based exercise challenge

The swimmers performed a sport-specific field-based exercise challenge following a low intensity warm up. Swimmers were required to maintain >80% maximum heart rate (220-age) for at least 8 minutes in order to optimally provoke EIB. Heart rate was measured objectively using pulse oximetry (M-pulse™, Merlin Medical, UK). FEV₁ (forced expiratory volume in 1 second) was measured using a Piko-6® portable spirometer (Ferraris Respiratory). Heart rate and FEV₁ were measured at baseline, immediately following challenge and at 5 and 10 minutes during recovery. A positive challenge was defined as a fall in FEV₁ of ≥10%.

Peak nasal inspiratory flow

Peak nasal inspiratory flow (PNIF) was measured using the In-Check® PNIF meter (Clement Clarke International Ltd, Harlow, UK). After horizontal positioning and restoration to zero, participants forcefully inhaled through their nose from residual volume to total lung capacity. The best of three measurements was taken.

Health questionnaire

A modified version of a questionnaire previously used in studies on elite athletes was used to assess the presence of exercise-induced symptoms [176]. Symptoms of chlorine-induced rhinitis were assessed using a visual analogue scale. Atopy was defined as a history of intermittent rhinoconjunctivitis (in

accordance with current ARIA definitions) and/or a positive skin prick test or/ IgE specific RAST test within the last 2 years.

Statistical analysis

SPSS version 15 (SPSS Inc, Chicago, Illinois) was used to perform the statistical analysis. Non-Gaussian data were log transformed prior to analysis. Normalized data were assessed using paired t-tests. A P value of less than 0.05 (two tailed) was considered significant. As this was a pilot observational study, no formal power calculation was used.

Results

Combined and free chlorine levels on the day were 1.66 and 0.3 mg/l respectively. Baseline characteristics are described in Table 13. Complete baseline data was available on 31/36 swimmers. Eight swimmers (22%) had known asthma. 18 (50%) had rhinitis according to the 2008 ARIA guidelines (11 = intermittent rhinitis, 7 = persistent rhinitis). There were no significant differences in FE_{NO} or N_{NO} pre vs post exposure: mean (95% CI) for FE_{NO} 17.9 (13.5 to 23.5) ppb vs 17.0 (12.8 to 22.5) ppb ($P = 0.12$); N_{NO} 47.5 (36.3 to 62.1) ppb vs 45.4 (34.0 to 60.6) ppb respectively ($P = 0.71$). Mean PNIF increased from 124.4 L.min⁻¹ (107.8 to 143.5) vs 136 L.min⁻¹ (121.3 to 152.5): ($P = 0.04$). Baseline FE_{NO} readings in asthmatics and non-asthmatics were 35.7 (8.57) and 18.5 (3.01) respectively.

13/36 (36%) of swimmers had a positive exercise challenge. 10/13 (77%) of these were not previously known to be asthmatic. 3/8 (38%) of asthmatic swimmers had a positive challenge. During the challenge swimmers achieved a mean (SEM) heart rate of 81.3% (1.5) maximum predicted.

36% (13) had a positive exercise challenge. 46% (6) of participants with a positive challenge were symptomatic, 54% (7) were asymptomatic. 42% (15) swimmers complained of worsening nasal symptoms post swimming, but only 13% (2) had a demonstrable fall in PNIF (mean fall 33 l/min), 87% (13) had no change. 9/15 (60%) had a pre-existing diagnosis of either intermittent or persistent rhinitis.

Table 13: Lower airway outcomes

Study no.	Age/Sex	Known Asthma	Asthma meds*	FEV ₁	% change FEV ₁ †	Baseline FE _{NO} (ppb)	Change in FE _{NO} (ppb)	Symptoms suggestive of EIB
1	16/M	N		6.28	-29.0	22	+1	Y
2	15/M	Y	L,A	4.65	-2.6	24	+5	N
3	16/M	N		5.57	-2.3	11	-2	Y
4	16/M	Y		5.27	-23.3	17	+1	N
5	15/M	Y	L,A,S	4.11	+9.0	28	+1	Y
6	21/M	Y	S,A	5.37	-6.1	76	-6	Y
7	15/F	N		4.26	-24.6	15	-1	N
8	15/F	N		4.27	-7.3	55	+17	N
9	13/F	N		3.44	-4.9	NA	NA	N
10	12/F	Y	A	3.63	-20.7	NA	NA	Y
11	18/M	N		3.51	-7.7	NA	NA	Y
12	15/M	N		6.32	-32.8	16	+1	N
13	15/M	N		4.56	-11.6	9	0	N
14	15/F	N		3.83	-14.1	12	-2	N
15	12/F	N		3.08	+7.5	22	0	N
16	12/F	N		2.70	+21.9	NA	NA	Y
17	13/F	N		3.26	-5.2	32	-11	Y
18	12/F	N		3.86	-14.2	13	NA	N
19	13/F	Y	S	3.10	+1.3	11	+1	Y
20	11/M	N		3.74	-0.8	13	+5	Y
21	14/F	N		3.11	+7.1	6	-1	Y
22	12/F	N		3.37	-4.2	10	-2	N
23	13/F	N		3.44	-17.2	17	-5	Y
24	11/M	N		2.85	-5.6	58	-23	Y
25	13/M	Y	S	3.24	+5.2	52	-3	Y
26	12/M	N		2.50	-0.8	6	-1	N
27	11/M	N		4.50	-30.4	10	-2	Y
28	12/M	N		3.19	-9.1	9	+6	Y
29	12/M	N		3.31	-3.3	12	0	N
30	12/M	N		2.32	-3.4	5	0	N
31	11/F	N		2.64	+1.5	13	-2	N
32	11/F	N		3.44	-11.6	11	-1	Y
33	11/F	Y	S	2.45	-17.1	42	-6	Y
34	11/F	N		3.15	-10.5	10	-2	N
35	12/F	N		2.88	+1.0	24	-2	N
36	11/F	N		3.16	-7.0	52	-1	N
Mean (SEM)	13.3 (0.38) 17M:18F	8 (22%)		3.73 (0.17)	-7.6 (1.96)	22.28 (3.20)	1.1 (1.10)	18 (50%)

* L= Leukotriene receptor antagonist, A= antihistamine, S= inhaled corticosteroid

† = maximal % change in FEV₁ observed during exercise challenge.

Table 14: Upper airway outcomes and training

Study no.	Known Rhinitis [†]	% fall PNIF	Change in N _{NO} (ppb)	History of Atopy	Decrease in nasal visual analogue score post exposure	Training (hr/wk)	Training (years)
1	I	0.0	11	Y	Y	15	10
2	I	-11.1	-72	N	N	12	5
3	P	+5.0	20	Y	Y	11	5
4	I	0.0	29	Y	Y	12	9
5	I	+58.8	38	Y	Y	14	8
6	P	+50.0	21	Y	Y	17	14
7	N	+100.0	-30	N	Y	12	7
8	N	+22.2	29	Y	N	12	6
9	N	+27.3	NA	N	N	9.5	3
10	N	NA	NA	Y	Y	11	4
11	I	NA	NA	Y	N	11	4
12	I	+50.0	-24	N	Y	14	2
13	N	-23.1	-38	N	Y	14	8
14	P	0.0	19	N	N	6	3.5
15	N	-31.6	-32	N	Y	12	4.5
16	N	NA	NA	N	N	9.5	5
17	P	+16.7	5	Y	N	10.5	3.5
18	I	NA	NA	Y	N	9.5	5.5
19	P	0.0	14	N	N	11	3.5
20	N	+8.3	46	N	N	11	3
21	I	-18.8	36	Y	N	12	9
22	N	+7.7	NA	N	N	13	7
23	P	+14.3	21	N	Y	12	2
24	N	+40.0	4	N	Y	5	3
25	P	0.0	51	N	N	11	6
26	N	0.0	-29	N	N	8	8
27	N	+11.1	18	N	N	7.5	2
28	N	+16.7	-16	N	Y	6	4
29	N	-5.9	26	N	N	6	5
30	N	+33.3	-1	N	N	7.5	4
31	N	+44.4	-5	N	N	6	3.5
32	N	-26.7	1	N	N	9	2
33	I	-9.1	-75	Y	N	5	6.5
34	N	+15.4	-20	N	N	6.5	3
35	I	0.0	-28	Y	Y	6	8
36	I	0.0	26	Y	Y	7	3.5
Mean (SEM)	I=11(31%) P=7(19%) N=18(50%)	+12.3 (4.88)	-1.5 (5.85)	14 (39%)	15 (42%)	10.2 (0.5)	5.3 (0.5)

[†] I= intermittent, P= persistent, N = none

Discussion

This study aimed to assess the combined effects of chlorine and exercise on the unified airway of adolescent elite swimmers. We found no significant change in either tidal (FE_{NO}) or nasal (N_{NO}) NO following a 2-hour training session, which included a high intensity anaerobic set. Using linear regression analysis, this change in NO was not influenced by asthma, presence of EIB or history of atopy. Furthermore, there was no correlation between upper and lower airway NO ($r=0.12$, $P=0.81$). We elected to use FE_{NO} as it is non-invasive, reproducible and easy to perform at the poolside. FE_{NO} is an established surrogate in asthma, which correlates closely with airway inflammation [60]. It is also used as a surrogate marker of inflammatory diseases in the upper airway [236], enabling us to examine effects on the unified airway. Additionally, FE_{NO} has been shown to rise in elite runners following a marathon [237]. The absence of any acute rise in FE_{NO} was therefore unexpected. Whilst forced vital manoeuvres are known to 'washout' FE_{NO} from the airways [194], there are no reports of exercise or hyperventilation decreasing FE_{NO} . Indeed, our results did not show any reduction post-swimming. It is also conceivable that the short duration of exposure was not sufficient to induce NO synthase, however, FE_{NO} has previously been shown to be elevated during an osmotic challenge suggesting that there is rapid induction of this enzyme [238]. We did not assess whether a late phase response was present in either the upper and lower airway. This is supported by anecdotal reports from swimmers who frequently complain of nasal congestion and symptoms, 4-6 hours after training.

The significant increase observed in PNIF post-swimming may not represent an improvement in inflammation, but paradoxically may signify a 'nasal douche effect'. Saline irrigation is known to significantly improve nasal congestion through a combination of improved mucociliary clearance and reduction of mucosal oedema [239]. 18 (50%) of swimmers had a pre-existing diagnosis of rhinitis, which is in line previously reported rates [240]. Rhinitis did not necessarily predict worsening of nasal symptoms post swimming, however, large-scale epidemiological studies are needed to investigate this issue further.

As part of this study we carried out a standardised field-based exercise challenge. Traditional field based testing in which athletes perform a challenge using their primary exercise, is usually limited by an inability to standardise both the cardiovascular workload and environmental conditions [177]. We therefore decided to use a standardised protocol based on the ATS (1999) guidelines for exercise challenge, using an objective measure of heart rate monitoring. In the current study, swimmers achieved an average heart rate of 81.3% of maximum, which was maintained for an average of 8 minutes.

We found a high prevalence (36%) of EIB in our cohort of adolescent elite swimmers. This is a unique finding, as previous figures are biased towards adult swimmers [172]. Indeed, 77% of our swimmers with a positive exercise challenge were not previously known to be asthmatic. Moreover, only 46% of swimmers

who were exercise-positive reported symptoms suggestive of EIB. The diagnosis of EIB based on symptoms alone, is known to be highly inaccurate [241]. This suggests that solely testing symptomatic athletes is grossly insufficient. At elite level small changes in physiological reserve could potentially translate into tangible improvements in performance. More importantly, adolescent swimmers with undiagnosed EIB may not reach their full potential, preventing them from progressing to compete at adult level. We therefore feel that universal screening of all elite swimmers for EIB and rhinoconjunctivitis and should be advocated.

We recognize some limitations in our study. It could be argued that the non-intensive exercise preceding the challenge may have induced a refractory period in some swimmers, which may have led to an underestimate of the true prevalence of EIB. However, previous studies have shown that low intensity warm-ups do not induce refractoriness prior to challenge [242]. 4/5 asthmatic swimmers with a negative exercise challenge were on inhaled corticosteroids, which may explain the low incidence of EIB in this group. Ideally, these would have been stopped; however, this would have interfered excessively with training. As expected FE_{NO} was elevated at baseline in asthmatics, but not in non-asthmatic swimmers. This is in keeping with previous studies. It is possible that the swimmers in this cohort were too young to develop major airway inflammatory changes. A larger study in a cohort of older more experienced adolescent swimmers would help eliminate this as a confounding factor. Whilst this study does not allow us to differentiate between the relative contributions of

exercise and chlorine on the airway we felt it was important to study swimmers in a “real life” situation.

Conclusions

In conclusion, we found that a 2 hr training session, including a high intensity anaerobic set, in a chlorinated indoor pool did not affect surrogate markers of inflammation in the unified airway. There was a high prevalence of undiagnosed EIB, highlighting the importance of screening of all elite swimmers for asthma.

Critique

In this study we were able to determine that there was a high prevalence of undiagnosed EIB in adolescent elite swimmers, highlighting the need for an effective screening test. Standardised field-based exercise testing in swimmers did appear to be a viable option, as environmental conditions in the pool were relatively constant. Although, one criticism, which was made during the presentation of this paper, was that a single low FEV₁ immediately post exercise did not effectively differentiate between EIB and exhaustion. We therefore made the decision to define EIB as a drop in FEV₁ >10% at two separate time points post exertion in subsequent studies.

The lack of response in FE_{NO} was somewhat surprising in view of the increase that had been reported in marathon runners. We hypothesised that we had perhaps missed a late phase response in airway inflammation, similar to that seen following allergen challenge. The decision was therefore made to measure this in a subsequent study. We also felt that it was important to compare field-based exercise challenge to the gold standard test (EVH). However, EVH requires a great deal of machinery, experienced technical staff and is not readily available at the poolside. We therefore decided to use mannitol challenge in our follow-up study, as this has been shown to correlate very closely with EVH.

Disconnect between standardised field based testing and mannitol challenge in Scottish elite swimmers

Introduction

Exercise-induced bronchoconstriction is defined as a transient increase in airway resistance that occurs after vigorous exercise [133]. Proposed aetiological mechanisms include airway drying and cooling as a consequence of increased ventilatory rates during strenuous exercise [243, 244]. Exercise is an indirect airway challenge which has been found to have a high level of specificity, but a low sensitivity for identifying EIB in cold weather and track athletes [179]. Sport-specific exercise in the field (FBT) is limited with respect to the standardization of both the workload and environmental conditions such as allergens, temperature, and humidity. Eucapnic voluntary hyperpnoea (EVH), a laboratory based challenge involving hyperventilation with dry air, has therefore been recommended as the 'gold standard' test for the diagnosis of EIB by the International Olympic Committee [175]. Mannitol, an osmotic challenge, has been found to correlate strongly with EVH [146].

Elite swimmers have higher rates of rhinoconjunctivitis and exercise induced bronchoconstriction (EIB) compared with any other groups of athletes [168, 231]. This may seem counterintuitive as they exercise in a warm moist environment. In a recent study Pedersen *et al* evaluated airway response in swimmers following four different challenge tests and found that EVH had a much lower sensitivity

than had been previously reported in other athletes [245]. Chlorine and chlorine metabolites are known to induce bronchial hyper-reactivity following accidental intense exposure [171]. Swimmers are repeatedly exposed to chlorine gases and their metabolites, which accumulate at the water/gas interface on the swimming pool surface. It has been reported that adult elite swimmers have significantly higher levels of airway inflammatory cells compared with healthy controls [172].

In a recent preliminary study of young swimmers attending the district level squad, we evaluated the acute response to chlorine and exercise using FE_{NO} , a well recognised surrogate of airway inflammation, and found no increase following a 2 hour training session [246]. We hypothesised that we may have missed a late phase response and therefore performed a follow up study in elite swimmers attending the national level training squad. In the present study we have evaluated the early and late phase FE_{NO} response in both the upper and lower airways. We have also compared the response to mannitol and sports specific physiologic exercise challenges.

Methods

Participants and study design

The Scottish National swimming squad (61 swimmers) were assessed over a three day residential training weekend at the National Swimming Academy in Stirling. Swimmers are subdivided into 2 squads: the national squad, and the national development squad. Swimmers underwent exhaled tidal (FE_{NO}) and nasal (N_{NO}) NO measurement, peak nasal inspiratory flow rate (PNIF), and FEV_1 before, immediately after, and 4-6 hours post swimming. A sport-specific exercise test was carried out during an intensive lactate set. All swimmers underwent mannitol challenge, and completed a health questionnaire. Challenge tests were incorporated into the training programme. Swimmers were asked to withhold anti-histamines, leukotriene receptor antagonists (LRTAs), nasal steroid sprays, and long acting β_2 - agonists (LABAs) for 1 week before the training session. Inhaled cortico-steroid inhalers could be taken as normal to avoid significant negative impact on training. Participants were asked to refrain from taking short acting β_2 -agonists (SABAs) 12 hours before the training weekend. This study was approved by the local ethics committee (REC ref: 09/S1402/6) and all participants gave written informed consent. Combined (free) chlorine levels on each of the three training days were: 1.66 (1.42), 1.58 (1.44), and 1.68 (1.40) mg/l, which are within UK recommended limits.

Demographics

Baseline characteristics are described in Table 15. The national squad were significantly older, trained more hours per week, over more years than the national development squad (table 16). Ten swimmers (17%) had physician diagnosed asthma, 3 were taking inhaled corticosteroids, one was taking a leukotriene receptor antagonist. 24 (40%) had rhinitis according to ARIA guidelines (16 = intermittent rhinitis, 8 = persistent rhinitis).

Table 15: Subject characteristics

	National squad (n=19)	National development squad (n=42)	All swimmers (n= 61)
Age (years)*	17.74 (0.51)	14.95 (0.15)	15.2 (0.25)
Physician diagnosed asthma	4 (21%)	6 (14%)	10 (17%)
Rhinitis	8 (42%)	16 (38%)	24 (40%)
Weekly training (hrs)*	19.39 (0.86)	15.45 (0.55)	16.69 (0.52)
Duration of competitive sport (years)*	8.47 (0.48)	5.95 (0.28)	6.74 (0.29)
Atopy	6 (40%)	7 (17%)	14 (24%)
Baseline FE _{NO} *	25.0 (1.1)	16.5 (1.1)	18.8 (0.2)
Positive FBT*	2 (13%)	7 (42%)	9 (16%)
Positive mannitol challenge*	5 (33%)	3 (8%)	8 (14%)

Data expressed as mean (SEM) unless otherwise stated.

*Significant difference between squads

Timing of tests

Complete baseline data were available on 60/61 swimmers. Exercise challenge was available in 57 (2 were injured and thereby unable to take part, and 2 did not stay for the full training camp). 59 swimmers carried out a mannitol challenge; one was unable to tolerate the procedure due to excessive coughing. Challenge tests were incorporated to fit into the training programme, consequently the National squad underwent mannitol challenge 5.01 (0.01) hours post exercise; whereas the development squad had their mannitol 18.28 (0.33) post exercise.

Immediate post swim measures were performed after a 2 hour swimming session. Delayed post swim measures were carried out 4.66 (0.18) hours after swimming. These were performed prior to mannitol challenge. During the challenge swimmers achieved a mean (SEM) heart rate of 85.0% (1.12) maximum predicted.

Measurements

FE_{NO}

All participants underwent measurement of FE_{NO} using a portable MINO (NIOX MINO® Airway Inflammation Monitor; Aerocrine AB, Solna, Sweden). A single reading was obtained in accordance with manufacturer's guidelines. Nasal exhaled NO was measured using an adapted MINO device, as previously described [235].

Sport-specific field-based exercise challenge

The swimmers performed a high intensity sport-specific field-based exercise challenge during an intensive lactate set. This was preceded by a standard low intensity warm up of approximately 15-20 minutes, to avoid muscular injury. During the high intensity lactate set, swimmers were required to maintain >80% maximum heart rate (220-age) for at least 8 minutes in order to optimally provoke EIB. Heart rate was measured objectively using pulse oximetry (M-pulse™, Merlin Medical, UK). FEV₁ (forced expiratory volume in 1 second) was measured using a Piko-6® portable spirometer (Ferraris Respiratory). Results from PIKO

devices have been found to be highly reproducible, and to correlate well with results from other portable and office based spirometers [247]. Heart rate and FEV₁ were measured at baseline, immediately following challenge and at 5 and 10 minutes during recovery. The best of two reproducible readings was utilised. A positive challenge was defined as a fall in FEV₁ of $\geq 10\%$ at ≥ 2 time points during recovery (in accordance with recommendations by drug-free sport UK for ATUE (therapeutic use exemption) certification)[174].

Mannitol challenge

Mannitol challenge was performed by administering spray-dry mannitol powder in gelatine capsule form (Aridol™ Pharmaxis Ltd, Sydney, Australia), inhaled from a dry powder device (Osmohaler, Pharmaxis Ltd. French's Forest, NSW, Australia) as previously described by Anderson and colleagues [136][183]. FEV₁ was measured 60 seconds after delivery of each dose (5, 10, 20, 40, 80, 160, 160, 160 mg) using a Piko-6® portable spirometer (Ferraris Respiratory). This device was used to ensure standardisation between both challenge tests. The test continued until the FEV₁ had fallen 15% or the maximal dose of 635 mg had been administered. The provoking dose of mannitol to cause a 15% fall in FEV₁ (PD₁₅) was calculated by log linear interpolation. A positive challenge was taken as being either a 15% fall in FEV₁ at the threshold dose (PD₁₅) or a 10% fall at successive time points.

Peak nasal inspiratory flow

Peak nasal inspiratory flow (PNIF) was measured using the In-Check® PNIF meter (Clement Clarke International Ltd, Harlow, UK). After horizontal positioning and restoration to zero, participants forcefully inhaled through their nose and the best of three measurements was taken.

Health questionnaire (symptoms)

A modified version of a questionnaire previously used in studies on elite athletes was used to assess the presence of exercise-induced symptoms [176]. Symptoms of chlorine-induced rhinitis (blockage/ running) were assessed using a visual analogue scale. Atopy was defined as a history of rhinoconjunctivitis (in accordance with current ARIA definitions) and/or a positive skin prick test or IgE specific RAST test within the last 2 years.

Statistical analysis

SPSS version 15 (SPSS Inc, Chicago, Illinois) was used to perform the statistical analysis. Non-Gaussian data were log transformed prior to analysis. Normalized data were assessed using repeated measures ANOVA with Bonferroni correction. Chi Squared test was used to check for associations in non-parametric data. Pearson's correlation was used for parametric data. A P value of less than 0.05 (two tailed) was considered significant. As this was an observational study, no formal power calculation was used.

Results

Challenge tests

8/59 (14%) of swimmers had a positive mannitol challenge 9/57 (16%) of swimmers had a positive exercise test. Only one swimmer was positive to both tests. There was no correlation between maximum FEV₁ drop in mannitol challenge and maximum FEV₁ drop in exercise challenge. 3/10 asthmatics were positive to mannitol, while only 2/10 were positive to exercise.

Results of subjects who achieved a $\geq 10\%$ fall in FEV₁ are detailed in table 16. 7/9 subjects deemed to have a positive exercise challenge had a fall immediately post exercise which was sustained at 5 minutes (7/7) and 10 minutes (5/7) recovery. The mean (SEM) %fall for these subjects immediately post exercise was 18.9 (7.0), and 15.2 (6.0) at 5 minutes. 3 subjects had a fall of $\geq 10\%$, which was not sustained; their mean (SEM) %fall was 11.1 (1.7) immediately and -0.47 (1.7) at 5 minutes recovery. Two subjects developed a sustained $\geq 10\%$ fall during recovery. The geometric mean (95% CI) mannitol PD₁₅ was 209.9 (128.7 – 342.3). The degree of bronchial reactivity, measured in terms of response-dose ratio (final % fall in FEV₁ divided by the total administered dose of mannitol), was similar in swimmers with EIB and those who did not respond to either test, 0.01 and 0.01 respectively. The response-dose ratio in those with a positive mannitol was higher at 0.06.

Table 16: % fall in FEV₁ in those with a fall \geq 10%

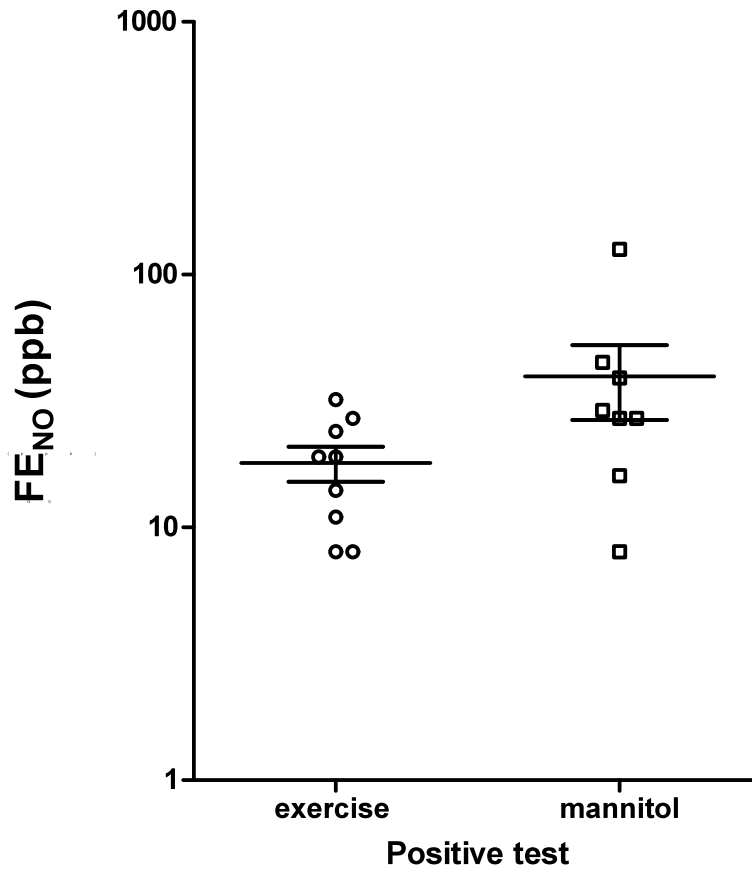
Time post exercise	Immediately post-exercise	At 5 minutes recovery	At 10 minutes recovery	Positive/ negative test
% Fall in FEV ₁	8.92	12.45	20.12	P
	-11.55	-0.19	10.06	N
	13.59	13.36	3.23	P
	29.25	23.03	22.61	P
	21.23	10.31	10	P
	26.03	13.7	9.13	P
	11.53	-5.9	-6.7	N
	18.61	14.93	14.52	P
	11.03	4.93	-2.82	N
	12.41	15.95	15.19	P
	11	15.16	11.98	P
	4.59	10.1	18.37	P
	10.67	-0.46	-2.09	N

P= exercise test considered to be positive, N= test considered negative

FE_{NO}

Baseline FE_{NO} was 26.7 (16.3 to 43.6) ppb in asthmatic swimmers and 17.4 (14.9 to 20.4) ppb in non-asthmatic swimmers (P=0.03). Swimmers with a positive mannitol had a significantly higher baseline FE_{NO} than those with a positive exercise challenge (figure 11). There was a significant association between positive mannitol challenge and baseline FE_{NO} >25 ppb ($X^2=7.76$, P=0.01). There was a weak correlation between maximal fall in FEV₁ during mannitol challenge and baseline FE_{NO} (r=0.26, P=0.03). There was no association between positive exercise and high baseline FE_{NO} ($\chi^2=0.127$, p=0.54). A significant decrease in FE_{NO} was observed pre vs. immediate and delayed post chlorine exposure: mean (95% CI) 18.7 (15.9 to 22.0) ppb vs. 15.9 (13.3 to 19.1) ppb (P <0.01), and 13.9 (11.5 to 16.7) ppb (P<0.01) respectively (table 17). This was irrespective of squad, atopic or asthmatic status.

Figure 11: Comparison of baseline FE_{NO} in patients with a positive exercise and positive mannitol challenge.



Presented as geometric mean and 95% CI

N_{NO}

There was no significant difference in N_{NO} between pre exposure, immediate, or delayed post exposure: 98.7 (5.4) ppb, 100.9 (5.0) ppb ($P = 0.70$), and 98.2 (4.5) (P=0.94) respectively (table 17).

PNIF

Mean PNIF increased from 142 L.min⁻¹ (6) at baseline to 162 L.min⁻¹ (6) immediately post exposure ($P < 0.01$). Delayed post exposure PNIF was not significantly different from pre-exposure readings ($P = 0.90$) (table 17). There was no significant increase in visual analogue scale at any point post exposure.

Table 17: Effects of swimming on FE_{NO} , N_{NO} and PNIF

	Pre-swim	Immediate post-swim	4-6 hrs post-swim
FE_{NO} (ppb) *	18.7 (15.9 - 22.0)	15.9 (13.3 - 19.1) ‡	13.9 (11.5 - 16.7) ‡
N_{NO} (ppb) †	98.68 (5.39)	100.93 (5.00)	98.22 (4.45)
PNIF (l/min) †	142 (6)	162 (6) ‡	143 (6)

* Shown as geometric mean (95% CI)

† Expressed as mean (SEM)

‡ Statistically significant change from pre-swim measure

Symptoms

43% (26) of swimmers complained of exercise related symptoms. 36% (8/22) of these had a positive exercise challenge (4 symptomatic swimmers were unable to take part in the challenge). 30% (7/26) of symptomatic swimmers had a positive mannitol challenge. 44% (4) of participants with a positive exercise challenge were symptomatic, 56% (5) were asymptomatic. 75% (6) of participants with a positive mannitol challenge were symptomatic, 25% (2) were asymptomatic.

Discussion

This study aimed to compare the response to standardised exercise testing and mannitol challenge in elite national level, swimmers. We also aimed to investigate the combined effects of chlorine and exercise on the unified airway. No association was found between mannitol and exercise challenge. 14% swimmers were positive to mannitol, 15% were positive to exercise challenge, while only one swimmer was positive to both. Whilst these findings are not supported by studies performed in cold weather or track athletes [177, 178], they are consistent with the findings of Pedersen *et al* who reported that EVH had a relatively low sensitivity in swimmers [245]. This is not entirely unexpected as the humidified air inspired by swimmers during exercise should decrease the rate of expired water loss during hyperpnoea. Despite this, swimmers have one of the highest prevalences of EIB, which would suggest that the theories of heat and water loss as a cause of EIB might not be totally applicable and other factors (such as the presence of chlorine) may be pertinent.

In the current study a disconnect was observed between AHR detected by standardised-FBT and mannitol challenge. Traditionally, FBT in athletes has been non-standardised and centred on a challenge using their primary exercise [177]. This will limit the ability to standardise both the cardiovascular workload and environmental conditions for repeated challenges or between athletes, making it inappropriate for direct comparison to highly standardised tests such as EVH or methacholine challenge. For example, a sprinter whose main event is a

50m freestyle will be unlikely to sustain the adverse effects on the airways for long enough to induce bronchoconstriction, however EIB in longer aerobic training sets may still have a significant effect on training and may still therefore be of importance to the swimmer. We therefore decided to use a standardised, rather than event specific protocol based on the ATS (1999) guidelines for exercise challenge and using an objective measure of heart rate monitoring [183]. In the current study all athletes were required to achieve an average heart rate of 80% of maximum, and maintain this for at least of 8 minutes. Environmental conditions within the pool over the 3-day period were constant. A positive test required the presence of a sustained fall in FEV₁ post exercise (i.e. a fall of $\geq 10\%$ at two time points post exercise) in order to exclude those who may have had a drop secondary to exhaustion.

In this study mannitol challenge was positively associated with high baseline FE_{NO}, whereas exercise challenge was not, suggesting that mannitol challenge may be more sensitive at picking out swimmers with a traditional 'inflammatory' asthmatic phenotype, rather than EIB. Leukotrienes are known to play a significant part in sustaining the contraction of airway smooth muscle and airway narrowing in EIB. It is therefore also of interest that mannitol positive swimmers, who have demonstrated that their airway is susceptible to endogenous release of leukotrienes, do not respond to exercise. This suggests that a different mechanism is responsible for the fall in FEV₁ with high intensity swimming. Mannitol challenge has previously been shown to correlate very closely with

eosinophils in induced sputum [248]. Swimmers are thought to have higher levels of eosinophils and neutrophils in their sputum [172]. However, closer inspection of the data by Helenius *et al* reveals that most swimmers with sputum eosinophila were in fact known asthmatics, whilst the greater majority of study population had eosinophil levels comparable to controls. Findings in participants without known asthma or AHR to methacholine (14/29) were not separately reported. A study by Boulet *et al* suggested that sputum neutrophils are up-regulated in swimmers following high intensity training [249]. This is of interest as asthmatic subjects with high sputum neutrophil counts have been found to have milder AHR to mannitol compared to those with a preponderance of other inflammatory cell types [250]. However, high sputum neutrophil counts would not serve to explain the disconnect observed between the two challenge tests.

In this regard, the current study used FE_{NO} as a surrogate of airway inflammation [194]. There was a significant fall in FE_{NO} following a 2-hour swim, and a trend towards a continuing drop at 4-6 hours post exposure. This was seen in both squads, and was not affected by asthmatic or atopic status. Forced vital manoeuvres are known to 'wash-out' FE_{NO} from the airways, however this effect has not been established in relation to exercise or hyper-ventilation [194]. Indeed, the continued decrease in FE_{NO} 4-6 hours post-exposure would not support a 'wash-out' effect and suggests another action. Case series describing airway changes following accidental high intensity chlorine exposure have described significant drops in FE_{NO} which persist for up to 2 months post-

exposure [171]. It has been suggested that acute chlorine exposure causes widespread oedema of the respiratory mucosa, degeneration and desquamation of the bronchial epithelium, resulting in exposure of vagal receptors and consequently enhanced vagal activity with bronchial hyper-reactivity [251]. Therefore, it seems plausible that repeated low-intensity exposure to chlorine may cause bronchial hyper-reactivity by a non-cellular inflammatory response. This would explain the high prevalence of EIB diagnosed by standardise-FBT, even in swimmers with normal baseline FE_{NO} . There have been several case reports of acute pulmonary oedema in swimmers and divers. In 2004 Adir et al published a case series of 70 swimmers with “swimming-induced acute pulmonary oedema” [252]. However, they reported clinical haemoptysis and a decrease in mean oxygen saturations to 76%. Adir and colleagues hypothesised that the pulmonary oedema may have been due to exercising in the prone position, as this will have affected the distribution of blood throughout the lung. Whilst no decrease in oxygen saturations was noted in our swimmers it is possible that a combination of exercising in the prone position and exposure to toxic metabolites may have caused a mild degree of airway oedema, which led to a decrease in bronchial flux. An alternative mechanism for the suppression of FE_{NO} is direct blockage of production through inhibition of NO synthase (the enzyme responsible for NO production).

Unlike FE_{NO} , there was no change in N_{NO} following a 2 hour swim. The cause for this is unclear, however, a possible explanation is that at exercise capacity

athletes mouth breath rather than breathing through their nose. Given this, the paranasal sinuses will have comparatively little gas exchange or chlorine exposure. There was also no change 4-6 hours post exposure, suggesting that there is no late phase response in NO. This is substantiated by the PNIF results which increase immediately post exposure but returns to baseline after 4-6 hours. The immediate improvement is most likely due to a 'nasal douche effect', given that saline irrigation is known to significantly improve nasal congestion [239]. There was additionally no significant change in visual analogue scale either immediately or 4-6 hours post-exposure. 27/60 swimmers had a PNIF ≤ 120 l/min at baseline. Given that national elite swimmers train on a daily basis during the season, it was not possible to establish a truly chlorine free baseline.

The diagnosis of EIB based on symptoms alone, is known to be highly inaccurate [241]. In the current study 43% of swimmers complained of exercise related symptoms. However, only 38% of these had a positive challenge, which is in keeping with the results of our previous study [246].

There were significant differences in baseline values between the two squads. The National squad (NS) were significantly older, trained more hours per week, over a greater number of years compared to the National Development squad (NDS). They also had a greater proportion of asthmatics, and a higher mean baseline FE_{NO} . It has been suggested that regular pool attendance is associated with an increased risk of asthma [253], which could explain why the older more

experienced team has a higher prevalence. However, this discrepancy may simply be due to the smaller number of swimmers in the NS.

We recognize some limitations in our study. Ideally both challenges would have been done on separate days; however, due to constraints of the training timetable, this was only possible for the national development squad. Edmunds *et al* found little evidence of a refractory period 4 hours post exercise [254], therefore the mean of 5.05hrs between challenges in the national squad should have been sufficient to re-establish stored inflammatory mediators. In addition, Anderson *et al* noted that exercising whilst breathing in warmed humidified air, did not induce mast cell degranulation, bronchoconstriction or not prevent the development of severe exercise induced asthma in response to a standard exercise challenge 30 minutes later [255]. These results are further substantiated by those of Hahn *et al* who found that exercise challenge with warm humidified air led to significantly lower fall in FEV₁ post challenge, and did not induce a refractory period [256]. The national development squad performed mannitol challenge 18 hours post exercise, therefore refractory period is not relevant in this group. Although, the national squad performed mannitol challenge 5 hours after exercise, only one swimmer had a positive exercise challenge subsequently followed by a negative mannitol challenge.

In the current study we measured FEV₁ for 10 minutes post exercise. It has been reported that peak fall in FEV₁ may occasionally occur up to 30 minutes [257].

However, the ATS guidelines on exercise testing conclude that “including a 30-minute post-exercise observation is controversial, because such a delay is infrequently seen” [183]. As a compromise, to minimise the interference with the athlete’s intensive training session, we decided that 10 minutes should be sufficient to pick up the majority of cases. It could additionally be argued that the non-intensive exercise preceding the challenge may have induced a refractory period in some swimmers, which may have led to an underestimate of the true prevalence of EIB. The low incidence of positive mannitol challenge in swimmers with a physician diagnosis of asthma was unexpected. Of those that were negative (7/10), 2 were taking regular ICS. Ideally, all preventer therapy would have been stopped prior to assessment, however it was felt that this would have interfered excessively with training. Of the remaining 5 swimmers, 2 had a high FE_{NO} (>30), and 3 had normal readings (<20). A normal FE_{NO} in the presence of a negative mannitol and exercise challenge suggests that these patients were wrongly diagnosed with asthma.

Conclusion

We have found no association between mannitol and standardised-FBT in elite swimmers. Mannitol challenge was associated with a high baseline FE_{NO} , however exercise challenge was not. This suggests that whilst mannitol may select swimmers with a 'traditional' inflammatory asthmatic phenotype, there may still be a role for a standardised field based challenge in high performance swimming, where the bronchoconstrictor response may have a more complex aetiology. Swimmers show a sustained fall in FE_{NO} following chlorine exposure suggesting that a non-cellular, perhaps neurogenic, type-inflammation may be important in airway dysfunction in this group of athletes. Further large scale studies are warranted to understand the pathophysiology of low dose chronic chlorine exposure in elite swimmers.

Critique

The results of this study were unexpected, and led us to realise that several potential aetiologies may be at play in elite swimmers, making the use of a single diagnostic test problematic. We recognise that further studies are required to confirm the existence of 'a swimming-specific bronchoconstrictor response', however such studies would be inherently difficult to perform. Ideally, standardised field-based challenge would be performed in a chlorine-rich and chlorine-free environment; unfortunately there are no chlorine-free pools in Scotland. If such a comparison were to be made, it would be important to find a group of elite swimmers who exercise solely in a chlorine-free environment, in order to exclude the effects of chronic low-grade chlorine exposure on the respiratory epithelium. It has been suggested that sea swimmers could constitute such a group, however such a comparison is problematic in that the focus of sea swimming is endurance whereas indoor swimming requires high intensity exercise of relatively short duration. In other words, elite indoor swimmers are therefore far more likely to exercise at the physiological extremes required to trigger EIB. Furthermore, the different strokes utilised in the two disciplines allow for the development of different muscle groups, making a direct comparison impossible. As an alternative, field-based challenge could be compared to standardised exercise challenge in a different discipline, e.g. CPET (which involves exercising on a treadmill or static cycle whilst breathing in air of a fixed temperature/ humidity). The difficulty with this is that swimmers develop certain muscle groups, and are frequently unable to maintain similar exercise intensities

whilst running or cycling. It has been suggested that 'chlorine challenge' could be employed, however as chlorine is known to induce reactive airways dysfunction syndrome (RADS) the ethics of this would have to be closely debated. It is likely that even in swimmers a battery of tests would need to be performed in order to determine the most appropriate test to monitor response in clinical trials.

Chapter 5:

Discussion and

conclusions

This work aimed to investigate potential mechanisms for improving the management of asthma utilising current available therapies. To this end the thesis is divided into two distinct sections: Establishing the role of surrogate markers of inflammation in clinical and research settings and tailoring treatment according to asthma phenotype.

It is estimated that as many as 300 million people of all ages, and all ethnic backgrounds, suffer from asthma, and that the burden of this disease to governments, health care systems, families, and patients is increasing [1]. Scotland has been reported to have the highest proportion of asthma per head of population worldwide, with 18.4% of inhabitants affected [1]. Worryingly, Scotland has been also ranked in the top third of countries with regard to asthma mortality, with 0.6 asthma deaths per 100 000 5-35 year olds [1]. This ranking is significantly higher than countries with lower GDPs such as Ecuador and Latvia, which is surprising, as >95% of the Scottish population have access to essential drugs [1]. National guidelines were introduced with the aim of standardising the management of asthma, however, the majority of asthmatics remain symptomatic. In 2006 an observational study of 9467 asthmatic patients, of all ages and clinical severities, in 319 general practices throughout Scotland was carried out [258]. They aimed to calculate the 'human cost' of asthma through the assessment of symptoms and lifestyle disruption, and to assess direct health care costs through a study of health service utilization. It was found that only 1/3 of patients were free from asthma symptoms. 4211 (66%) had experienced

symptoms due to asthma in the past month; with 12% (770) reporting lost time from work/school due to asthma. In the preceding 12 months 20% (1916) had experienced an acute exacerbation, 321 (3%) patients had attended an Accident and Emergency (A&E) department because of an asthma related problem, 455 (5%) had attended out patients, and 237 (2.5%) were admitted to hospital. Fifteen of these patients experienced a total of 28 days stay in an intensive care unit. These results indicate that the human and economic costs of asthma remain a substantial burden to the Scottish population. It is clear that a new approach to asthma management is urgently required.

Currently asthma therapy is titrated on the basis of symptoms and markers of airflow limitation, both of which are known to correlate poorly with the underlying asthmatic inflammation. Strategies in which asthma therapy has been targeted to suppress surrogate markers of inflammation have proved very successful [37, 38]. However, applying this strategy to a wider 'community' setting has proved problematic. The main difficulties lie with the identification of an appropriate surrogate marker of inflammation. Both sputum eosinophils and methacholine bronchial challenge are time consuming to perform and require the presence of highly trained staff and expensive machinery, making them impractical for use in the community. FE_{NO} was a promising candidate, especially following the development of the portable MINO device. However, a recent community based study by Menzies et al found that FE_{NO} was an insensitive method (sensitivity, 66.7%; specificity, 51.9% at a cutoff value of 20 ppb) for identifying patients who

subsequently exacerbated [76]. This was likely due to frequently exacerbating patients receiving higher doses of maintenance inhaled corticosteroids, leading to suppression of FE_{NO} . Bronchial challenge is a well-established surrogate marker of inflammation. The development of a challenge test utilizing dry powder mannitol offers an exciting opportunity, as only a portable spirometer and dry power inhaler are required [136]. In this respect, the first study to be included in this thesis looked at the use of mannitol challenge to identify changes in airway inflammation during community-based down titration of asthma medication. Establishing patients on the lowest dose of inhaled steroids, in order to reduce the risk of dose related side effects, is recommended in current guidelines, but rarely carried out in clinical practice. In this study we were able to demonstrate that mannitol challenge can easily and safely be used in a community setting. In addition we showed that a significant reduction in ICS dose could be achieved in a without any worsening of airways inflammation, lung function, or quality of life. 34% of subjects demonstrated an improvement (and 41% showed no change) in AHR post step-down, suggesting that compliance improved on entry into the clinical trial. Only a minority of patients showed a worsening of AHR, which would be expected if subjects were initially concordant with treatment. A rate of 19% is consistent with concordance rates reported in previous studies [197]. Treatment of asthmatic patients in primary care has been driven by results obtained from randomised controlled trials, during which treatment compliance is reinforced. These therefore, do not reflect a real life scenario. This study highlights the potential pit falls of extrapolating data to a primary care setting where compliance

rates may be poor. Perhaps there is a need for the development of more effective strategies which support asthmatics to use established therapies correctly rather than continue a culture of escalating therapy, without frequent review and step-down if appropriate. Improvement of asthma care in the community may require an approach that includes more effective supervision and targeted assessment to enhance adherence and reduce airways inflammation. Further studies are required to assess the impact of such a strategy in a prospective manner. Large randomised control trials are required to further evaluate the use of mannitol challenge as a tool by which to titrate inhaled therapy in a community setting.

The importance of identifying appropriate surrogate markers of inflammation is not restricted to clinical practice, and is equally important in the research setting. This is particularly true when determining therapeutic equivalence between inhaled products. A successful therapeutic equivalence study requires demonstration of a significant dose-response relationship with at least two doses of the test compared with, if possible, two doses of the reference product [259]. An ideal clinical efficacy study design for establishing a dose response needs to be sensitive (i.e. steep-dose-response slope), reproducible, have low inter- and intra-subject variability, and be achievable with low patient numbers. Current EMEA guidelines state that the most well-used study design is the double-blind, randomised, parallel group comparison of test and reference product [259]. However, this design requires the careful matching of cases and controls, which

is problematic due to the inherent heterogeneity of asthma. The alternative is a crossover study, which has the advantages of allowing cases to act as their own controls, and allows the use of a much smaller population, however this requires the use of a reliable, repeatable endpoint measure. In addition, the lack of a carry-over effect much be demonstrated. The EMEA currently recommend that the primary efficacy variable should be a pulmonary function measure, preferably FEV1 measured regularly, if possible daily at home or at least every two weeks in the clinic [259]. Alternative primary variables can be considered but their sensitivity to detect differences between adjacent doses of inhaled corticosteroid must be demonstrated. The purpose of utilizing inhaled steroids in the management of asthma is to suppress the underlying airway inflammation. It therefore appears nonsensical to select a marker that is distant from this process. The aim of the second study was therefore to determine which inflammatory outcome measures provided sufficient assay sensitivity, as part of a crossover design, for detecting dose response effects on airway and systemic markers. Methacholine challenge was selected as the primary outcome variable as significant dose separation has previously been reported with 100 μ g vs. 500 μ g fluticasone propionate [213]. In this respect, we also demonstrated sensitivity of the methacholine bioassay, as there was a significant dose-response relationship for both formulations. The common slope for the overall log dose-response relationship was highly significant, demonstrating that the doses selected coincided with the steep part of the dose-response curve for BHR. The 95% CIs for the relative dose potency ratio of HFA vs. CFC were close to unity at

1.10, however, the 95%CI (0.49–2.66) was outside predetermined equivalence limits of $\pm 50\%$ (0.5-2.0) [212]. Current EMEA guidelines state that bioequivalence in respect of systemic exposure can be demonstrated if the 90% confidence interval is entirely contained within 80 – 125%. However, the FDA also accept a $\pm 33\%$ limit (i.e. 0.67-1.5) for PD studies. Whilst we accept that these limits are valid when comparing across doses (e.g. 200ug HFA vs CFC or 800ug HFA vs CFC), such limits are not appropriate for calculating relative potency from the slope of the DRC from a Finney bio-assay. We have found, from previous experience, that it is not possible to achieve confidence intervals within these extremely tight boundaries for pharmacodynamic endpoints [260]. In a previous study from our department involving 27 patients we found that the 95% confidence interval for the relative dose potency of two formulations of budesonide was 0.5-2.46. In order to increase the power in the present study we doubled the number of patients, and utilized methacholine challenge as opposed to adenosine-5-monophosphate (AMP) challenge, as this is considered to yield lower variability. The decision to aim for a relative potency of 0.5 – 2 from Finney was based on this study [212]. However, even with more than double the number of patients, lower equivalence limits were not achieved.

Several studies have suggested that FE_{NO} may be able to discriminate doses of ICS [69, 71, 81, 261] however; significant limitations in their study design put these results into question. For example, a number of these studies utilized a cumulative-dose design, suggesting that the observed dose–response

relationship may have been due to a time effect rather than a dose effect. In the current study a significant dose response relationship was seen for both HFA and CFC, however, the 95% confidence interval was also outside of predetermined equivalence limits. In this study, cumulative dose was also an issue for FE_{NO} , however the same cannot be said for methacholine challenge. Sovijvari et al demonstrated that the doubling dilution difference between FP and placebo was 1.19, 1.33 and 1.27 after 72 hours, 2 weeks and 4 weeks respectively [214]. Thereby demonstrating that near-maximal effects on methacholine PC_{20} are obtained within the first two weeks of treatment. In the present study we observed PC_{20} shifts of 1.55dd and 2.16dd following 200 μ g and 800 μ g of HFA budesonide, giving a mean difference of 0.51dd which is too large to be simply due to a time effect. The current evidence suggests that the tight equivalence limits recommended by both the EMEA and FDA cannot be achieved in PD studies, without the recruitment of hundreds of participants. Among the study designs that have been examined to date, a crossover design utilizing methacholine challenge as the primary endpoint seems to hold the most promise; but even for this primary endpoint, more than doubling of the study subjects, did not achieve the required confidence intervals. This suggests that wider confidence interval will have to be accepted if bioequivalence is to be demonstrated using pharmacodynamic endpoints.

These two studies have demonstrated that the selection of appropriate surrogate markers of inflammation can potentially improve the clinical management of

asthma and play an important role in asthma research. Clearly the titration of steroid therapy in asthma is of vital importance, however, it must be noted that no two individuals have the same response to the same dose of the same steroid. This is in effect a reflection of the degree of steroid responsiveness, or resistance, and any understanding of the individual's responsiveness to proposed steroid therapy should allow improved therapeutic options, tailored to the individual. It is likely that there is a spectrum of steroid responsiveness in asthma, with rare complete resistance at one end, but a relative resistance in patients who require high doses of inhaled or oral corticosteroids [262]. True (complete) corticosteroid resistance is very rare, with an estimated prevalence of 1:1000 asthmatics [262]. The reasons for the variability in individual therapeutic responses to drugs used to treat asthma are complex, involving both genetic and environmental factors as well as levels of adherence with therapy [263, 264]. One of the most influential environmental factors is cigarette smoking, which is known to significantly worsen asthma control through a combination of epithelial toxicity, oxidative damage, and recruitment of inflammatory cells [152, 153]. In addition, smokers with chronic asthma are less sensitive to the beneficial effects of both inhaled and oral corticosteroids compared with nonsmokers with asthma [157, 158, 160]. Therapeutic studies, which form the basis for current asthma guidelines, often exclude current smokers due to concerns about recruiting participants with chronic obstructive pulmonary disease (COPD). Consequently information on which drugs are most appropriate to treat smokers with asthma is severely lacking. Knowing how best to manage this group of patients is of

considerable importance because smoking is common among patients with asthma, current smoking rates among asthmatic patients have been reported as ranging from 17-35% [148, 150, 151, 161, 162]. Although smoking cessation has been shown to improve asthma control, lung function and lead to a decline in sputum neutrophil counts many asthmatics are either unable or unwilling to stop smoking [217]. The lack of available evidence means that guidelines do not recommend alternative treatment strategies for smokers, other than suggesting that 'higher doses of inhaled steroids may be required' [8].

Only a handful of studies to date have investigated this issue. In 2007, Lazarus and colleagues compared the response to low-dose inhaled steroid (becomethasone 400mcg daily) with an oral leukotriene receptor antagonist (montelukast 10mg daily) for 8 weeks in smokers and non-smokers [265]. Their main findings were that inhaled steroid significantly increased pre-bronchodilator FEV1 in non-smokers only, whereas montelukast increased morning peak flow in smokers only. Unfortunately, these changes were only seen within group and not between groups. Furthermore, neither montelukast nor beclomethasone lead to improvements in either sputum eosinophils or methacholine challenge. A more recent study by Spears and colleagues investigated the potential additive effects of theophyllines on ICS [266]. The recruitment of histone deacetylase (HDAC) is known to partly mediate the anti-inflammatory actions of corticosteroids. HDAC is a nuclear enzyme involved in the switching off of activated inflammatory genes [267]. Cigarette smoke has been shown to reduce HDAC activity in vitro [268],

which could explain corticosteroid insensitivity seen in smokers with asthma. In vitro studies have shown that HDAC activity can be restored by low-doses of theophylline [269]. Spears et al therefore set out to determine whether theophyllines, in combination with low-dose ICS had any additive effects on lung function over ICS or theophyllines alone [266]. They found that subjects treated with the combination demonstrated significant improvements in morning PEF and mean pre-bronchodilator FEV1. These improvement were noted to be superior to those seen with montelukast [265] and high dose inhaled steroid [159]. However, no differences were observed in sputum inflammatory cells, other than a decrease in lymphocyte count, the clinical significance of which was unclear. In our study we investigated the benefits of combination therapy with LABA and ICS over doubling the dose of inhaled steroid in smokers and non-smokers, and were able to demonstrate an improvement in lung function similar to that seen in non-smokers. Furthermore, smokers appeared to gain proportionally greater benefit from the addition of salmeterol than non-smokers on methacholine AHR. These results support the observation made by Pedersen and colleagues during a post-hoc analysis of the Gaining Optimal Asthma control (GOAL) study' that smokers would need 18 years less treatment, by taking FPSM vs. FP alone, to prevent an exacerbation than non-smokers [163]. In our study we found no significant benefit, in any primary or secondary outcome, from high dose inhaled FP (1000ug/day) in smokers, which goes against current recommendations that smokers may benefit from higher doses of ICS [8]. This assumption was based on a study by Tomlinson et al, who demonstrated that high dose ICS decreased

peak flow variability to a similar degree in both smokers and non-smokers [159]. Peak flow, like spirometry, reflects airway caliber and as such is relatively distant from the underlying asthmatic inflammatory process [21]. In this respect we saw no significant improvement in either peak flow dairy cards or ACQ in smokers. Whilst the results of our study suggest that smoking asthmatics gain no benefit from inhaled steroids, we would not advocate that they be treated with salmeterol alone. The use of LABAs as monotherapy has been shown to lead to an increase in asthma related deaths [270]. Further safety research would be required prior to making such a declaration. Similarly, although our results suggest similar improvements in spirometry to those seen during smoking cessation, we would still advocate smoking cessation as the first line intervention in smoking asthmatics. The detrimental effects of smoking are not solely limited to poorer asthma control, significant increases in cardiovascular risk and increased risk of malignancy dictate that smoking cessation be discussed on a regular basis.

We had hoped to provide a mechanistic explanation for this improvement by including both methacholine and mannitol challenge. The intention was that mannitol would provide a sensitive marker of inflammation, whereas methachline would provide a measure of airway tone. Interestingly, similar results were seen with both challenge tests (although differences between treatments in smokers did not reach significance with mannitol). We would have expected to see a greater improvement in mannitol PD₁₅ following treatment with double dose FP compared to FPSM, as was observed during a similar study which utilised AMP

challenge [271]. In view of the fact that salmeterol has been shown to have no meaningful in vivo activity we have to hypothesise that the improvements conferred by LABAs was either due to their stabilizing effect on airway smooth muscle [225] and mast cells [272], or to their ability to preserve the integrity of the respiratory epithelium [227]. These findings suggest that mannitol may be less may be less specific for inflammation than originally thought. Further studies are required to determine the exact mechanism of action of this indirect bronchial challenge test. However, despite this, we have successfully demonstrated that alternative, treatment approaches tailored to different subgroups of asthmatics may be beneficial.

Another group of individuals in whom the traditional approach to asthma management may not be appropriate is elite athletes. It has been well documented that the mechanism of bronchoconstriction in athletes involves the drying and/or cooling of airways [134]. However, even within this category there are athletes to which this mechanism of action is unlikely to apply. Swimmers, for example, exercise in a warm, humid environment and are therefore not subject to the same environmental strains as cold weather or endurance athletes. It therefore seems unlikely that tests designed to reproduce hyperosmolar shifts will have the same diagnostic sensitivity in swimmers as they do in these other groups of athletes. The aim of the fourth and fifth studies included in this thesis was therefore to compare various diagnostic tests in elite swimmers, in order to determine which were the most sensitive. This would allow us to determine which

test would be most specific for future use in studies monitoring benefit from medical therapy.

We hypothesised that exposure to chlorine had to play an important part in the underlying pathogenesis of EIB in swimmers, as traditional explanations did not fit, and wanted to determine the effect of chlorine and exercise on the unified airway prior to undertaking studies comparing the different tests. Adult elite swimmers have been shown to have significantly higher levels of airway inflammatory cells compared with healthy controls [172]. We therefore conducted a pilot study to assess the combined effects of chlorine and exercise on the unified airway of adolescent elite swimmers, including estimating the prevalence of rhinoconjunctivitis and EIB. We elected to use FE_{NO} as it is a non-invasive, reproducible, well-established marker of airway inflammation, which can be used to assess both the upper and lower airways [236]. In addition, FE_{NO} had been shown to rise following a marathon [237]. The absence of any acute rise in either tidal or nasal FE_{NO} post exercise challenge was therefore unexpected. We hypothesised that we may have missed a late phase response, similar to that seen during allergen challenge. This assumption was supported by anecdotal reports from swimmers who complained of nasal congestion and symptoms, 4-6 hours after training. The use of a standardised field based test was a novel approach. Traditional field based testing, in which athletes perform a challenge using their primary exercise, was previously limited by an inability to standardise both the cardiovascular workload and environmental conditions [177]. We

therefore decided to use a standardised protocol based on the ATS guidelines for exercise challenge, using an objective measure of heart rate monitoring. Using this test we were able to identify a high prevalence (36%) of EIB in our cohort of adolescent elite swimmers.

The next step was therefore to compare our standardised exercise test to mannitol challenge, as a surrogate for the gold-standard test, EVH. We found a disconnect between the challenge tests, in that 14% swimmers were positive to mannitol, 15% were positive to exercise challenge, while only one swimmer was positive to both. Whilst these findings were not supported by studies performed in cold weather or track athletes [146, 177, 178], they were consistent with the findings of Pedersen *et al* who reported that EVH had a relatively low sensitivity in swimmers [245]. Interestingly, swimmers with a positive mannitol challenge had a significantly higher baseline FE_{NO} , than those who were positive to exercise, suggesting that mannitol challenge may be more sensitive at picking out swimmers with a traditional 'inflammatory' asthmatic phenotype, rather than EIB. When we looked at FE_{NO} we found that there was a significant decrease post exercise, which was maintained 4-6 hours post exercise. Case reports of children exposed to high doses of chlorine have shown increased bronchial hyper-reactivity and persistently low FE_{NO} levels. We therefore hypothesised that a similar pathogenic mechanism may be at play in the swimmers, a sort of 'swimmer-specific bronchoconstrictor response' thereby explaining why they didn't respond to both challenges. Interestingly, similar results have been

reported previously. Bonsignore et al compared the effects of a 5km race on spirometry and FE_{NO} in competitive outdoor pool swimmers and sea swimmers. Although the chlorine levels in the outdoor pool were extremely low, they noted a significant decrease in FE_{NO} post-exercise, which was not observed in sea swimmers. No differences were seen in sputum inflammatory cell composition, and no changes in FEV_1 were noted post exercise, however, again the field-based challenges were not standardised [273]. Furthermore, neither group was considered to be 'elite', indicating that perhaps the intensities of exertion required to trigger EIB were not reached.

Unfortunately, subsequent publications do not correlate these results. Castricum et al recently published a study comparing standardised field-based challenge, EVH and lab-based challenge in 33 adult swimmers [274]. They found that only 1 of the 33 subjects had a positive field swim challenge compared to 18 with a positive EVH challenge, and 4 with a positive laboratory cycle challenge. Only 1 of the 33 subjects was positive to all 3 challenges. Whilst the disconnect between the challenge tests reflects our results, the low prevalence of EIB diagnosed by field challenge is somewhat surprising. The prevalence of EIB diagnosed in both studies presented in this thesis were 36% and 15% respectively. Rates in the first study may have been artificially high as positive challenge was defined as a single >15% drop in FEV_1 post exercise. In the second study these criteria were tightened to >15% drop at two time points post exercise, with the aim of excluding those with a drop secondary to exhaustion. In the study by Castricum

et al the field-based challenge took place in an ozone filtered pool, which would have resulted in significantly lower concentrations of chlorine. Further studies are therefore required to compare standardized field based challenge in high and low chlorine environments.

Much controversy surrounds the diagnosis of EIB itself. It has been suggested that EIB in elite athletes is simply a reflection of very mild asthma, which only becomes symptomatic due to extremes of exercise. Whilst this is almost certainly true in a subgroup of athletes, such as the swimmers who were positive to mannitol in the 5th study, I believe that EIB occurs as a distinct clinical entity. Exercise is the most common trigger of bronchospasm in those who are known to be asthmatic, affecting between 50 and 90% of all individuals with asthma [275]. However, EIB also occurs in up to 10% of subjects who are not known to be atopic or asthmatic [276]. Elite athletes claim that exercise is the most prominent trigger of asthma symptoms, and rarely complain of diurnal variation or nocturnal symptoms [241]. The pathogenesis of asthma-like symptoms in elite athletes is likely to be multifactorial, and is not completely understood. However, during competitive sport the use of full lung capacity means that large volumes of atmospheric air that is cold, dry and polluted are inhaled on a regular basis. This overcomes the ability of the upper airways to warm up and humidify the air reaching the smaller airways [277], bringing about airway narrowing through water and heat loss [134]. These airway differences, together with some degree of inflammation, lead to EIB or “sports asthma” [278]. Several studies have

suggested that athletes have different patterns of airway inflammation compared to 'traditional inflammatory' asthmatics [279-281]. Furthermore, unlike traditional asthma, EIB and airway inflammation have been shown to attenuate on cessation of competitive sport [173]. However, no study to date has excluded subjects with either a physician diagnosis of asthma or 'typical' asthma symptoms (e.g. nocturnal waking or diurnal variation). It is therefore unclear whether subjects who are responsive to exercise have a different pattern of inflammation than those who are responsive to direct challenge, or what role inflammation actually plays in the pathogenesis of EIB. Karjalainen et al carried out a study in which elite skiers were classified according to their response to methacholine challenge [279]. They found that all skiers, irrespective of challenge, had significantly higher neutrophil counts on bronchial biopsy than asthmatic controls. However, methacholine-responsive skiers had significantly higher eosinophil counts than non-responders, suggesting that methacholine challenge may select out those with more traditional 'inflammatory' asthma [279]. Unfortunately, this study did not compare response to exercise between the two groups. Lab-based studies have shown that exposing healthy subjects to cold air whilst running can induce an increase in the number of granulocytes and macrophages in the BAL [282]. Repetitive hyperpnoea itself has been shown to recruit eosinophils to the airways and to promote the release of inflammatory cytokines [283]. Although inflammatory cells have been documented in the airways of non-asthmatic subjects with EIB, it should be stressed that their role in the development of airway hyper-responsiveness is currently unknown. Smooth

muscle dysfunction on the other hand undoubtedly plays a large role in the pathogenesis of traditional asthma, hence why bronchial challenge tests to direct stimuli have such a strong negative predictive value. The same however, cannot be said for EIB, and direct challenge has been shown to be a poor diagnostic tool for EIB in athletes [176, 179], adding yet further weight to the argument that a different pathogenesis is at play in this group of individuals.

The current gold standard test for the diagnosis of EIB is EVH. However, as a positive results is seen in up to 50% of athletes [179, 245], one has to wonder whether it is over-diagnosing EIB? Whilst the lack of environmental standardisation makes field-based testing a relatively poor diagnostic tool in track and cold weather athletes, the same cannot be said for elite swimmers. Which begs the question: What is the clinical significance of a positive EVH challenge in a swimmer who doesn't bronchoconstrict during a standardised exercise challenge? In other words, is EVH simply identifying a normal response at the extremes of physiology? Further research is require to investigate the role of inflammatory cells in the pathogenesis of EIB, and to investigate whether athletes who bronchoconstrict to exercise, EVH or direct challenge have different inflammatory cell compositions or airway dynamic effects.

Conclusions

1. The use of inflammatory surrogates has opened many possibilities in both the fields of clinical management and asthma research. We have demonstrated that mannitol challenge can safely be used in the community, and that down-titration of inhaled steroid is possible without a significant rise in airway inflammation. These findings suggest that an approach that includes effective supervision and targeted assessment, to enhance adherence and reduce airways inflammation, may be beneficial.
2. Methacholine challenge has sufficient assay sensitivity to be used as the primary outcome measure in crossover studies examining the bioequivalence of inhaled corticosteroids. However, wider confidence intervals will have to be accepted if bioequivalence is to be demonstrated using pharmacodynamic endpoints.
3. Fluticasone/salmeterol combination affords more bronchoprotection, and greater bronchodilation than doubling the dose of fluticasone in smoking asthmatics. This study adds further weight to the argument that smoking asthmatics require a different approach to treatment than their non-smoking counterparts.
4. Mannitol challenge may not be as specific for inflammation as originally thought, as it appears to be attenuated by LABA induced smooth muscle stabilisation.

5. Adolescent elite swimmers have a high prevalence of asthma and rhinitis.
6. Swimmers are not exposed to the same environmental strains as other cohorts of elite athletes, and the pathogenesis of their EIB is likely to be different. The disconnect observed between swimmers with high FE_{NO} and a positive mannitol, and those with a positive field-based challenge, suggests that a 'swimmer specific bronchoconstrictor response' may be at play. This is substantiated by the persistent drop in FE_{NO} observed 4-6 hours post exercise.

Publications arising from this thesis

Clearie K, Vaidyanathan S, Williamson P.A, Goudie A, Short, P, Lipworth, B.J.
Effects of chlorine and exercise on the unified airway in adolescent elite Scottish swimmers. Allergy 2010 Feb; 65(2): 269-73

Clearie KL, Williamson PA, Vaidyanathan S, Short P, Goudie A, Burns P, Hopkinson P, Meldrum K, Howaniec L, Lipworth BJ. ***Disconnect between standardized field-based testing and mannitol challenge in Scottish elite swimmers.*** Clinical Experimental Allergy. 2010 May;40(5):731-7.

Clearie KL, Jackson CM, Fardon TC, Williamson PA, Vaidyanathan S, Burns P, Lipworth BJ ***Supervised step-down of inhaled corticosteroids in the community – an observational study.*** Respiratory Medicine. 2010 (epub ahead of print).

Clearie KL, Williamson PA, Meldrum K, Gillen M, Carlsson LG, Carlholm M, Ekelund J, Lipworth BJ. ***Pharmacokinetic and Pharmacodynamic Comparison of Hydrofluoroalkane and Chlorofluorocarbon Budesonide.*** British Journal of Clinical Pharmacology. 2010 (epub ahead of print)

Poster presentations arising from this thesis

Disconnect between standardised field based testing and mannitol challenge in Scottish elite swimmers.

- ***Association of Physicians of Great Britain and Ireland (APAM)
Annual Meeting (Dundee, 16th April 2010)***

Establishing equivalence of inhaled corticosteroids using pharmacokinetic and pharmacodynamic outcomes

- ***Scottish Thoracic Society meeting (Stirling, October 2009)***
- ***European respiratory Society meeting (Barcelona, September 2010)***

Effects of chlorine and exercise on the unified airway in young elite Scottish Swimmers.

- ***Scottish Thoracic Society meeting (Scone, April 2009)***
- ***American Thoracic Society meeting (San Diego, May 2009)***
- ***European Thoracic Society meeting (Vienna, September 2009)***

Supervised step-down in a community setting does not increase airways inflammation in mild-to-moderate asthmatics.

- ***American Thoracic Society meeting (San Diego, 16th May 2009)***

Fluticasone/Salmeterol combination confers significant benefits in smoking asthmatics

- ***European respiratory Society meeting (Barcelona, September 2010)***
- ***British Thoracic Society meeting (London, December 2010)***
- ***Scottish Thoracic Society meeting (Stirling, October 2010)***

Oral Presentations arising from this thesis

Disconnect between standardised field based testing and mannitol challenge in Scottish elite swimmers.

- ***British Thoracic Society meeting (London, December 09)***
- ***Scottish Thoracic Society meeting (Stirling, October 09)***

Supervised Step-down In a Community Setting Does Not Increase Airways Inflammation In Mild-to-Moderate Asthmatics

- ***British Thoracic Society meeting (London, December 09)***
- ***Scottish Thoracic Society meeting (Stirling, April 08)***

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